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APPLICATION FOR UNITED STATES PATENT

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Invention: PROTEIN L AND HYBRID PROTEINS THEREOF

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SPECIFICATION

Protein L and Hybrid Proteins Thereof

The present invention relates to sequences of protein L which bind to light chains of immunoglobulins. The invention also relates to hybrid proteins of protein L having the ability to bind to light chains of all Ig and also to bind to light and heavy chains of immunoglobulin G, DNA-sequences which code for the proteins vectors that contain such DNA-sequences, host cells transformed by the vectors, methods for preparing the proteins, reagent apparatus for separating and identifying immunoglobulins, compositions and pharmaceutical compositions which contain the proteins.

The invention relates in particular to the DNA-sequence and to the amino acid sequence of the light-chain forming domains of protein L.

Proteins which bind to the constant domains (of high affinity) of the immunoglobulins (Ig) are known. Thus, protein A (from Staphylococcus aureus) (Forsgren, A. and Sjöquist, J. (1966) Protein A from Staphylococcus aureus. I. Pseudo-immune reaction with human gamma-globulin. J. Immunol. 97: 822-827) binds to IgG from various mammal species. The binding of protein A to IgG is mediated essentially via surfaces in the Fc-fragment of the heavy chain of the IgG-molecule, although a certain bond is also effected with surfaces in the Fab-fragment of the IgG. Protein A lacks the ability of binding to human IgG3 and neither will it bind to IgG from several other animal species, such as important laboratory animals, for instance rats and goats, which limits the use of protein A.

Protein G (Björck, L. and Kronvall, G. (1984) Purification and some properties of streptococcal protein G, a

novel IgG-binding reagent. J. Immunol. 133: 969-974; Reis, K., Ayoub, E. and Boyle, M. (1984) Streptococcal Fc receptors. I. Isolation and partial characterization of the receptor from a group C streptococcus. J. Immunol. 132: 3091-3097) binds to heavy chains in human IgG and to all four of its subclasses and also to IgG from most mammals, including rats and goats.

Protein H (Åkesson, P., Cooney, J., Kishimoto, F. and Björck, L. (1990) Protein H - a novel IgG binding bacterial protein. Molec. Immun. 27: 523-531) binds to the Fc-fragment in IgG from human beings, monkeys and rabbits. However, the bond is weaker than in the case of protein G and A, which may be beneficial when wishing to break the bond with a weak agent, for instance when purifying proteins which are readily denatured with the aid of antibodies.

Protein M (Applicant's Patent Application PCT/SE 91100447) binds to the Fc-fragment in IgG from humans, monkeys, rabbits, goats, mice and pigs.

Protein L (Björck, L. (1988) Protein L, a novel bacterial cell wall protein with affinity to Ig L chains. J. Immunol. 140: 1194-1197), which binds to the light chains in immunoglobulins from all of the classes G, A, M, D and E is known (USP 4,876,194). The amino acid sequence and the binding domains of this protein, however, have hitherto been unknown.

The aforesaid proteins can be used in the analysis, purification and preparation of antibodies and for diagnostic and biological research.

The elimination of immunoglobulins, with the aid of

plasmapheresis, can have a favourable effect on some autoimmune diseases. A broadly binding protein would be an advantage when wishing to eliminate all classes of antibodies in this context.

It has long been known that infectious conditions can be prevented or cured with the introduction of an immune serum, i.e. a serum which is rich in antibodies against the organism concerned or its potentially harmful product. Examples hereof are epidemic jaundice, tetanus, diphtheria, rabies and generalized shingles. Antibodies against a toxic product may also be effective in the case of non-infectious occasioned conditions. Serum produced in animals against different snake venoms is the most common application in this respect. However, the administration of sera or antibody preparations is not totally without risk. Serious immunological reactions can occur in some cases. Singular cases of the transmission of contagious diseases, such as HIV and hepatitis through the agency of these products have also been described. In order to avoid these secondary effects, it has been desirable to produce therapeutic antibodies in test tubes. A large number of novel techniques for the preparation of antibodies in test tubes have been proposed in recent years. Examples of such techniques are hybridom techniques, synthesis of chima-antibodies and the preparation of antibodies in bacteria. These techniques also enable antibodies to be specially designed which can further widen the use of such molecules as therapeutics, for instance in the case of certain tumour-diseases. In the case of some of these novel methods, however, the product totally lacks the Fc-fragment to which all of the described IgG-binding proteins, with the exception of protein L, bind. There is consequently a need of a process for purifying anti-

bodies for therapeutic use, wherein proteins which have a broad binding activity/specificity, can be of value.

It has long been possible to utilize the antibody reaction with its high grade specificity for diagnosing past or, in some cases, ongoing infections with different parasites. This indirect method of indicating infectious agents is called serology and, in many cases, may be the only diagnostic alternative. In certain cases, it can also be of interest to exhibit specific IgE- or IgA-antibodies. When diagnosing with the aid of serology, the antigen is most often fastened to a solid phase, whereafter serum taken from the patient is incubated with the antigen. Antibodies that have been bound from the patient can then be detected in different ways, often with the aid of a secondary antibody (for instance, an antibody which is directed against the light chains of human antibodies) to which an identifiable label has been attached, such as alkaline phosphatase, biotin, radioactive isotopes, fluorescein, etc. In this context, a protein having a broad Ig binding capacity can be used as an alternative to secondary antibodies.

There are a number of non-therapeutic and non-diagnostic reasons for the necessity to bind antibodies. Antibodies are often used in research, both for detection and for purifying the antigen against which they are directed. All techniques which facilitate the purification of antibodies and, in particular, techniques which enable different classes to be purified, are of interest in this context.

Consequently, there is a serious need of a protein which has a broad binding activity/specificity and which binds to several different classes of immunoglobulins from different animal species. At present, there is no known

protein which will bind to all immunoglobulin classes. The earlier known proteins A, G, H and M bind only to heavy chains in IgG. The known protein L (Björck et al, 1988) binds to the light χ-chains and γ-chains in immunoglobulins of all classes, although the bonds are much weaker on the κ-chains. Applicant has charted protein L, has determined the amino acid sequence for protein L, has identified the light-chain binding domains on protein L, and has used these to produce hybrid proteins which possess the IgG-Fc-binding domains of protein G. The Applicant is able to show through protein LG that a protein of broader binding activity/ specificity can be produced thereby. The aforesaid proteins A, G, H and M bind to the same surfaces, or to very closely lying surfaces on IgG-Fc. The protein L which binds to light chains can thus be combined with any other functionally similar protein which binds to the Fc-fragment of heavy chains. A similar broadening of the Ig-binding activity is achieved with all alternatives.

Thus, the present invention relates to the sequence of protein L which binds to light chains in Ig and has the amino acid sequence disclosed in Figure 1, and variants, subfragments, multiples or mixtures of the domains B1-B5 having the same binding properties. The invention also relates to a DNA-sequence which codes for such protein sequences, for instance the DNA-sequence in Figure 1.

The invention is concerned with a hybrid protein which is characterized by comprising domains which bind to the light χ-chains and λ-chains in immunoglobulins of all classes, and also comprises domains which bind to heavy chains in immunoglobulin G, wherein those domains which bind to the light chains are chosen from among the B1-, B2-, B3-, B4- and B5-domains in protein L (see Claim 1)

and those domains which bind to heavy chains of immunoglobulins are chosen from the C1-, C2- and C3-domains in protein G; the A-, B- and C1-domains from protein H; the A-, B1-, B2- and S-domains in protein M1 or the E-, D-, A-, B- and C-domains in protein A (see Figure 6) and variants, subfragments, multiples or mixtures of these domains that have the same binding properties which bind to heavy chains of immunoglobulins.

By subfragment is meant a part-fragment of the given domains or fragments which include parts from the various domains having mutually the same binding properties. By variants is meant proteins or peptides in which the original amino acid sequence has been modified or changed by insertion, addition, substitution, inversion or exclusion of one or more amino acids, although while retaining or improving the binding properties. The invention also relates to those proteins which contain several arrays (multiples) of the binding domains or mixtures of the binding domains with retained binding properties. The invention also relates to mixtures of the various domains of amino acid sequences having mutually the same binding properties.

The invention relates in particular to a hybrid protein designated LG, and is characterized in that the hybrid protein includes the B-domains in protein L which bind to the light chains in immunoglobulins, and the C1-domains and C2-domains in protein G which bind to heavy chains and have the amino acid sequence disclosed in Figure 3. The invention also relates to variants, subfragments, multiples or mixtures of these domains.

Protein LG is a hybrid protein having a molecular weight of about 50 kDa (432 amino acids) and comprising four domains, each of which binds to light chains in immuno-

globulins, and two IgG-binding domains from protein G. The hybrid protein combines a broad IgG-binding activity, deriving from the high-grade binding ability of protein G to the Fc-fragment of the heavy chain on IgG with the ability of the protein L to bind to light chains of all classes of immunoglobulins. Thus, protein LG binds polyclonal human IgG, IgM, IgA, IgD and IgE. The affinity for human polyclonal IgG is $2 \times 10^{10} M^{-1}$. All four human immunoglobulin classes are bound. Binding to human IgG is effected with both the κ-and the λ-chain. Both the Fc-fragment and the Fab-fragment of IgG are bound to the hybrid protein. The protein also binds human IgA-, IgD-, IgE- and IgM-antibodies. The bond is stronger to human immunoglobulins which carry χ than to those which carry the λ-isotope of light chains. IgG from most mammals will be bound by protein LG, thus also IgG from goats and cows, which do not bind to protein L. However, rabbit-IgG which binds relatively weakly to protein L will bind well to the fusion protein. IgM and IgA-antibodies from mice, rats and rabbits will be bound to the protein.

Protein LG is highly soluble. It is able to withstand heat and will retain its binding properties even at high temperatures. The binding properties also remain in a broad pH-range of 3-10. The protein withstands detergent and binds marked or labelled proteins subsequent to separation in SDS-PAGE and transference to membranes with elektroblotting. The protein can be immobilized on a solid phase (nitrocellulose, Immobilon®, polyacrylamide, plastic, metal and paper) without losing its binding capacity. The binding properties are not influenced by marking with radioactive substances, biotin or alkaline phosphatase. (The binding abilities of the protein LG are disclosed in Example 3).

The protein comprises 432 amino acids and has a molecular weight of 50 kDa deriving therefrom. The sequence is constructed of an ala sequence of the three last amino acids in the A-domain of the protein L (val-glu-asn), this ala sequence being unrelated to the two proteins, whereafter the four mutually high-grade homologous B-domains from protein L follow. The first of the B-domains is comprised of 76 amino acids, and the remaining domains are each comprised of 72 amino acids. The first nine amino acids from the fifth B-domain are included and followed by two non-related amino acids (pro-met). The protein G-sequences then follow. The last amino acid in the so-called S-domain from protein G is followed by an IgG-binding domain from protein G (C1; 55 amino acids), the intermediate D-region (15 amino acids) and the second IgG-binding C-domain (C2; 55 amino acids). The last amino acid is a methionine, which occurs in natural protein G as the first amino acid in the so-called W-region.

The invention also relates to DNA-sequences which code for the aforesaid proteins.

The gene which codes for the IgG-binding amino acid sequences can be isolated from the chromosomal DNA from Staphylococcus aureus based on the information on the DNA-sequence for protein A (S. Löfdahl, B. Guss, M. Uhlen, L. Philipsson and M. Lindberg. 1983. Gene for staphylococcal protein A. Proc. Natl. Acad. Sci. USA. 80: 697-701) and Figure 6, or from G-streptococcus, preferably strain G 148 or C-streptococcus, preferably strain Streptococcus equisimilis C 40, based on the information on protein G (B. Guss, M. Eliasson, A. Olsson, M. Uhlen, A.-K. Frej, H. Jörvall, I. Flock and M. Lindberg. 1986. Structure of the IgG-binding

regions c streptococcal protein G. EMBO. J. 5: 1567-1575) and Figure 6, or from group A-streptococcus, e.g. S. pyogenes (type M1) based on the information on the DNA-sequence for protein H (H. Gomi, T. Hozumi, S. Hattori, C. Tagawa, F. Kishimoto and L. Björck. 1990. The gene sequence and some properties of protein H - a novel IgG binding protein J. Immunol. 144: 4046-4052) and Figure 6, or from the chromosomal DNA in group A-streptococcus type M1 based on the information on the DNA-sequence for protein M (Applicant's Patent Application, PCT/SE 91100447) and Figures 6 and 7. The gene which codes for the protein that binds to light chains can be isolated from the chromosomal DNA from Pepto-coccus magnus 312 based on the information on the DNA-sequence for protein L in Claim 2.

By using the chromosomal DNA't obtained from the aforesaid bacteria as a template, a DNA-fragment defined with the aid of two synthetic oligonucleotides can then be specifically amplified with the aid of PCR (Polymerase Chain Reaction). This method also enables recognition sites to be incorporated for restriction enzymes in the ends of the amplified fragments (PCR technology, Ed: PCR Technology. Principles and Applications for DNA Amplification. Ed. Henry Erlich. Stockton Press, New York, 1989). The choice of recognition sequences can be adapted in accordance with the vector chosen to express the fragment or the DNA-fragment or other DNA-fragments with which the amplified fragment is intended to be combined. The amplified fragment is then cleaved with the restriction enzyme or enzymes concerned and is combined with the fragment/the other fragments concerned and the fragments are then cloned together in the chosen vector (in this case, the expression vector) (Sambrook, J.E. Fritsch and T. Maniatis, 1989, Molecular cloning: A laboratory manual, 2nd Ed. Cold Spring Harbor Laborato-

ries, Cold Spring Harbor, New York, USA). The plasmid vector pHD313 can be used (Dalböge, H.E. Bech Jensen, H. Töttrup, A. Grubb, M. Abrahamscn, I. Olafsson and S. Carlsen, 1989. High-level expression of active human cystatin C in Escherichia coli. Gene, 79: 325-332), alternatively one of the vectors in the so-called PET-series (PET 20, 21, 22, 23) retailed by Novagen (Madison, Wisconsin, USA).

The hybrid proteins are then incorporated in an appropriate host, preferably E. coli. The invention also relates to such hosts as those in which the hybrid proteins are incorporated.

Those clones which produce the desired proteins can be selected from the resultant transformants with the aid of a known method (Fahnestock et al., J. Bacteriol. 167, 870 (1986).

When the proteins that can bind to the light chains in the immunoglobulins and to the heavy chains in IgG have been purified from the resultant positive clones with the aid of conventional methods, the binding specificities of the proteins are determined for selection of those clones which produce a protein that will bind to the light chains in immunoglobulins and to the heavy chains in IgG.

Subsequent to having isolated plasmid DNA't in said clone with conventional methods, the DNA-sequence in the inserted material is determined with known methods (Sanger et al., Proc. Natl. Acad. Sci. USA 74, 5463 (1977).

The invention also relates to DNA-sequences which hybridize with said identified DNA-sequences under conven-

tional conditions and which code for a protein that possesses the desired binding properties. Strict hybridizing conditions are preferred.

Expression of the genes can be effected with expression vectors which have the requisite expression control regions, the structural gene being introduced after said regions. As illustrated in Figure 1 and Claim 2, the structural gene can be used for protein LG or other hybrid proteins with protein L.

With regard to expression vectors, different host-vector-systems have been developed, of which the most suitable host-vector-systems can be selected for expression of the genes according to the present invention.

The present invention also relates to a method of producing the inventive hybrid proteins by cultivating a host cell which is transformed with an expression vector in which DNA't which codes for the proteins according to the invention is inserted.

This method includes the steps of

(1) inserting into a vector a DNA-fragment which codes for the hybrid proteins;

(2) transforming the resultant vector into an appropriate host cell;

(3) cultivating the resultant, transformed cell for preparation of the desired hybrid protein; and

(4) extracting the protein from the culture.

In the first step, the DNA-fragment which codes for the hybrid protein is inserted in a vector which is suitable for the host that is to be used to express the hybrid protein. The gene can be inserted by cleaving the vector with an appropriate restriction enzyme, and then legating the gene with the vector.

In the second step, the vector with the hybrid plasmid is inserted into host cells. The host cells may be Escherichia, coli, Bacillus subtilis or Saccharomyces cerevisiae or other suitable cells. Transformation of the expressions hybrid vector into the host cell can be effected in a conventional manner and clones which have been transformed can then be selected.

In the third step, the obtained transformants are cultivated in an appropriate medium for preparation of the desired proteins by expression of the gene coded for the hybrid protein.

In the fourth step, the desired protein is extracted from the culture and then purified. This can be achieved with the aid of known methods. For instance, the cells can be lysed with the aid of known methods, by treating the cells with ultrasonic sound, enzymes or by mechanical degradation. The protein which is released from the cells or which excretes in the medium can be recovered and purified with the aid of conventional methods often applied within the biochemical field, such as ion-exchange chromatography, gel filtration, affinity chromatography with the use of immunoglobulins as ligands, hydrophobic chromatography or reverse-phase chromatography. These methods can be applied individually or in suitable combinations.

As before mentioned, the inventive proteins may be used for binding, identifying or purifying immunoglobulins. They can also be bound to pharmaceuticals and used in formulations which have delayed release properties. To this end, the protein may be present in a reagent appliance for pharmaceutical composition in combination with appropriate reagents, additives or carriers.

The proteins can be handled in a freeze-dried state or in a PBS-solution (phosphate-buffered physiological salt solution) pH 7.2 with 0.02% NaN_3. It can also be used connected to a solid phase, such as carbohydrate-based phases, for instance CNBr-activated sepharose, agarose, plastic surfaces, polyacrylamide, nylon, paper, magnetic spheres, filter, films. The proteins may be marked with biotin, alkaline phosphatase, radioactive isotopes, fluorescein and other fluorescent substances, gold particles, ferritin, and substances which enable luminescence to be measured.

Other proteins may also be used as carriers. These carriers may be bound to or incorporated in the proteins, in accordance with the invention. For instance, it is conceivable to consider the whole of proteins A, G, H, M as carriers for inserted sequences of protein L which bind to light chains. In turn, these carriers can be bound to the aforesaid carriers.

The pharmaceutical additions that can be used are those which are normally used within this field, such as pharmaceutical qualities of mannitol, lactose, starch, magnesium stearate, sodium saccharate, talcum, cellulose, glycose, gelatine, saccharose, magnesium carbonate and similar extenders, such as lactose, dicalcium phosphate and the like; bursting substances, such as starch or derivatives thereof; lubricants such as magnesium

stearate and the like; binders, such as starch, gum aribicum, polyvinylpyrrolidone, gelatine, cellulose and derivatives thereof, and the like.

The invention will now be described in more detail with reference to the accompany drawings, in which

Figure 1 illustrates the plasmid pHD389; the ribosomal binding sequence, the sequence for the signal peptide from **ompA** and recognition sequence for several restriction enzymes are shown;

Figure 2 illustrates the amino acid and nucleic acid sequence for protein LG;

Figure 3 is a schematic overall view of the production of protein L;

Figure 4 is a schematic overall view of the production of protein LG;

Figures 5a, 5b and 5c are schematic overall views of the production of the hybrid proteins LA, LM and LH respectively;

Figure 6 is a schematic inclusive illustration of protein A, G, H and M1. IgGFc-binding domains are for protein A: E, D, A, B and C; for protein G: C1, C2 and C3; for protein H: A and/or B; and for protein M1: A, B1, B2, B3 and S;

Figure 7 illustrates the amino acid and nucleic acid sequence for protein M1;

Figure 8 illustrates Western Blot for protein G, L and LG with certain immunoglobulins and immunoglulubulin fragments; and

Figure 9 illustrates Slot-Blot for protein L, G and LG with IgG, Igχ and Ig Fc.

The amino acid and nucleic acid sequence of the light-chain binding domains of protein L is illustrated in Claims 1 and 2 respectively.

It will be observed that the drawings are not to scale.

Example 1

Cloning and expression of the IgG-light-chain-binding domains in Protein L

Construction of synthetic oligonucleotides (primers) for amplifying sequences coded for protein L, domain B1-B4

It has been found that a protein L peptide (expressed in E. coli) constructed of the sequence ala-val-glu-asn- domain B1 (from protein L) binds to the light chains of the immunoglobulins (W. Kastern, U. Sjöbring and L. Björck. 1992. Structure of peptostreptococcal protein L and identification of a repeated immunoglobulin light chain-binding domain. J. Biol. Chem. in-print). Since this simple protein L-domain has a relatively low affinity to Ig, (1×10^{7} M^{-1}), and since the naturally occurring protein L which is constructed of several mutually similar domains (B1-B5) has a high affinity to Ig (1×10^{10} M^{-1}) four of these domains have been expressed together in the following way:

PL-N and PL-C1 are synthetic oligonucleotides (manufactured by the Biomolecular Unit at Lund University (Sweden) in accordance with Applicant's instructions) which have been used to amplify a clonable gene fragment which is amplified with PCR (Polymerase Chain Reaction) and which codes for four Ig-binding protein L domains (ala-val-glu-asn-B1-B2-B3-B4-lys-lys-val-asp-glu-lys-pro-glu-glu). Amino acids in the protein L-sequence are given for the primer which corresponds to the coded strand (PL-N):

PL-N: 5'-GCTCAGGCGGCGCCGGTAGAAAATAAAGAAGAAACACCAGAAAC-3'
valgluasnlysglugluthrproglu

5'-end of this oligonucleotide is homologous with the coded strand in the protein L-gene (emphasized): those codons which code for the last three amino acids in the A-domain (val-glu-asn) are followed by the codons for the first six amino acids in the first of the Ig-binding domains in protein L (B1).

PL-C1: 5'-CAGCAGCAGGATTCTTATTATTCTTCTGGTTTTTCGTCAACTTT
CTT-3'

This oligonucleotide is homologous with the opposing non-coding strand in the gene for protein L (the sequence corresponds to the first nine amino acids in domain B5).

DNA-fragments which have been amplified with the aid of PL-N contain the recognition sequence for the restriction enzyme **HpaII** (emphasized) immediately before the codon which is considered to code for the first amino acid (val) in the expressed protein L-fragment. The fragment which is cleaved with **HpaII** can be ligated with

DNA (in this case, consisting of the used expression vector pHD389) which has been cleaved with the restriction enzyme **NarI**. The DNA-fragment that has been cleaved with **HpaII** and ligated with vector pHD389, which has been cleaved with **NarI**, will be translated in the correct reading frame. The construction results in translation of an additional amino acid (ala) immediately in front of the first amino acid in protein L.

DNA-fragments which have been amplified with the aid of PL-C1 will contain the recognition sequence for the restriction enzyme **BamHI** (overlined above the sequence) immediately after the sequence which codes for the last amino acid in the expressed protein L-fragment (glu). The vector pHD389 contains a unique recognition sequence for **BamHI** as part of its so-called multiple cloning sequence which follows the **NarI** recognition sequence. DNA-fragments which have been amplified with the aid of PL-C1 will include two so-called stop-codons (emphasized) which results in translation of the fragment inserted in the vector to cease.

The sequence which was considered to be amplified contains no internal recognition sequences for the restriction enzymes **HpaII** or **BamHI**.

Amplifying and cloning procedures

(PCR) (Polymerase Chain Reaction) was effected with a protocol described by Saiki, R.D. Gelfand, S. Stoffel, S. Scharf, R. Higuchi, G. Horn, K. Mullis and H. Erlich, 1988; Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487-49127; PCR was effected in a Hybaid Intelligent Heating-block (Teddington, UK): 100 µl of a reaction mixture contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM

$MgCl_2$, 100 µg/ml gelatine, 300 µM with respect to each of the deoxynucleotides (dATP, dCTP, dGTP, dTTP), (Pharmacia), 20 pmol of each of the oligonucleotides PL-N and PL-C1, 10 µl of a target (template) DNA-solution containing 0.1 mg/ml of chromosomal DNA from Peptostreptococccus magnus, species 312. The mixture was covered with mineral oil (Sigma) and DNA't was denatured by heating to 98°C for 10 minutes. 2.5 units of AmpliTaq (Perkin Elmer Cetus, Norwalk, CT) were added and PCR was then carried out with 25 cycles consisting of a denaturing step at 94°C for 1 minute, followed by a hybridizing step at 56°C for 1 minute, and finally by an extension step at 72°C for 1 minute. Amplified DNA was analyzed by electrophoresis in agarose gel. The amplified DNA't was cleaved with the restriction enzymes **HpaII** (Promega), (8 units/µg amplified DNA) and **BamHI** (Promega), (10 units/µg amplified DNA) at 37°C. The thus amplified and subsequently cleaved DNA-product was isolated by electrophoresis in a 2% (weight by volume) agarose gel (NuSieve agarose, FMC Biproducts) in a TAE-buffer (40 Mm Tris, 20 Mm Na-acetate, 2 Mm EDTA, Ph 8.0). The resulting 930 base-pair large fragment was cut from the gel. The DNA concentration in this removed gel-piece was estimated to be 0.05 mg/ml. The agarose-piece containing the cleaved, amplified fragment was melted in a water bath at 65°C, whereafter the fragment was allowed to cool to 37°C. 10 µl (0.5 µg) of this DNA was transferred to a semimicrotube (Sarstedt) preheated to 37°C, whereafter 1 µl of the vector pHD389 was immediately added and cleaved with **NarI** (Promega) and **BamHI**, 1 µl 10xligas-buffer (Promega and 1 µl T4 DNA-ligase (Promega; 1 unit/µl). The ligating reaction was then used to transform E. coli, strain LE392, which had been competent in accordance with the rubidium/calcium-chloride-method as described by Kushner (1978). Molecular biological standard methods have been used in the manipulation

of DNA (Sambrook, J.E. Fritsch and T. Maniatis, 1989. Molecular cloning: A laboratory manual. 2nd Ed. Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, USA). The cleaving and ligating conditions recommended by the manufacturer of DNA-ligase and restriction enzymes have been followed in other respects.

Expression system

The vector pHD389 (see Figure 2) is a modified variant of the plasmid pHD313 (Dalböge, H.E. Bech Jensen, H. Töttrup, A. Grubb, M. Abrahamson, I. Olafsson and S. Carlsen, 1989. High-level expression of active human cystatin C in Escherichia coli. Gene, 79: 325-332). The vector, which is replicated in E. coli (contains ori = origin of replication from plasmid pUC19) is constructed so that DNA-fragments which have been cloned into the cleaving site of **NarI** will be transcribed and translated downstream of and in the immediate vicinity of the signal peptide (21 amino acids), from envelope-protein ompA from E. coli. Translation will be initiated from the codon ATG which codes for the first amino acid (methionine) in the signal peptide. This construction permits the translated peptide to be transported to the periplasmic space in E. coli. This is advantageous, since it reduces the risk of degradation of the desired product of enzymes occurring intracellularly in E. coli. Moreover, it is easier to purify peptides which have been exported to the periplasic space. Unique recognition sequences (multiple cloning sequences) for several other restriction enzymes, among them **ecoRI**, **SalI** and **BamHI** are found immediately after the **NarI** cleaving site. An optimized so-called Shine-Dalgarno-sequence (also called ribosomal binding site, RBS) is found seven nucleotides upstream from the ATG-codon in the signal sequence from **ompA**, this optimized sequence binding to a

complementary sequence in 16S rRNA in the ribosomes and is responsible for the translation being initiated in the correct place. The transcription of such DNA as that which is co-transcribed with the signal sequence for ompA is controlled by the P_R-promotor from coliphage λ. The vector also contained the gene for cI857 from coliphage λ whose product down-regulates transcription from P_R (and whose product is expressed constitutively). This cI857-mediated down-regulation of transcription from P_R is heat-sensitive. The transcription regulated from this promotor is terminated with the aid of a so-called rho-independent transcription terminating sequence (forms a structure in DNA't which results in the DNA-dependent RNA-polymerase leaving the DNA-strand) which is placed in the vector immediately downstream of the multiple cloning sequence. The plasmid also carries the ß-lactamase gene (from the plasmid pUC19) whose product permits ampicillin-selection of E. coli clones that have been transformed by the vector.

Selection of protein L-producing clones

The transformed bacteria are cultivated, or cultured, on culture plates with an LB-medium which also contained ampicillin in a concentration of 100 µg/ml. Cultivation of the bacteria progressed overnight at 30°C, whereafter the bacteria were transferred to an incubator where they were cultivated for a further 4 hours at 42°C. The plates were kept in a refrigerator overnight. On the next day, the colonies were transferred to nitrocellulose filters. Filters and culture plates were marked so as to enable the transferred colonies to be readily identified on respective culture plates. The culture plates were again incubated overnight at 30°C, so that remaining rests of transferred bacteria colonies could again grow. The plates were then kept in a refrigerator.

The bacteria in the colonies on the nitrocellulose-impressions were lysed by incubating the filter in 10% SDS for 10 minutes. Filters containing lysed bacteria were then rinsed with a blocking buffer which comprised PBS (pH 7.2) with 0.25% gelatine and 0.25% Tween-20 (four baths, 250 ml each at 37°C), whereafter the filter was incubated with radioactively marked (marked with ^{125}I in accordance with the chloramin-T-method) Ig-κ-chains (20 ng/ml in PBS with 0.1% gelatine). The incubation took place at room temperature over a period of 3 hours, whereafter non-bound radioactively marked protein was rinsed-off with PBS (pH 7.2) containing 0.5 M NaCl, 0.25% gelatine and 0.25% Tween-20 (four baths, 250 ml each at room temperature). All filters were exposed to X-ray film. Positive colonies were identified on the original culture plate. Clones which reacted with Ig-κ-chains were selected and analyzed with respect to the size on the DNA-fragment introduced in the vector. One of these clones was selected for the production of protein L, pHDL. The DNA't introduced from this clone into plasmid pHD389 was sequenced. The DNA-sequence was found to be in full agreement with corresponding sequences (B1-B4 and 21 bases in B5) in the gene for protein L from Peptostreptococcus magnus, strain 312. The size and binding properties of the protein produced by clone pHDL was analyzed with the aid of SDS-PAGE (see Figure 8), dot-blot experiment (see Figure 9) and competitive binding experiments.

Production of protein L

Several colonies from a culture plate with E. coli pHDL were used to inoculate a preculture (LB-medium with an addition of 100 mg/l ampicillin), which was cultured at 28°C overnight. On the following morning, the preculture was transferred to a larger volume (100 times the volume

of the preculture) of fresh LB-medium containing ampicillin (100 mg/l) and was cultured in shake-flasks (200 rpm), (or fermentors) at 28°C. The culture temperature was raised to 40°C (induction of transcription) when the absorbency value at 620 nm reached 0.5. Cultivation then continued for 4 hours (applied solely to cultivation in shake-flasks). Upon completion of the cultivation process, the bacteria were centrifuged down. The bacteria were then lysed with an osmotic shock method at 4°C (Dalböge et al., 1989 supra). The lysate was adjusted to a pH = 7. Remaining bacteria rests were then centrifuged down, whereafter the supernatent was purified on IgG-sepharose in accordance with earlier described protocol for protein G and protein L (U. Sjöbring, L. Björck and W. Kastern. 1991. Streptococcal protein G: Gene structure and protein binding properties. J. Biol. Chem. 266: 399-405; W. Kastern, U. Sjöbring and L. Björck. 1992. Structure of peptostreptococcal protein L and identification of a repeated immunoglobulin light chain-binding doman. J. Biol. Chem. in-print.

The expression system gave about 20 mg/l of protein L when cultivation in shake-flasks. The culture was deposited at DSSM, Identification Reference DSSM E. coli LE392/pHDL.

Example 2

Cloning and expression of protein LG

Construction of oligonucleotides (primers) for amplifying sequences which code for protein LG

Protein L

It has been found that a protein L-peptide (expressed in E. coli) constructed of the sequence ala-val-glu-asn-domain B1 (from protein L) will bind to the light chains of the immunoglobulins (Kastern, Sjöbring and Björck, 1992, J. Biol. Chem. in-print). Since the affinity of this simple domain to Ig is relatively low (1×10^{-7} M^{-1}) and since the naturally occurring protein L, which is comprised of several mutually similar domains (B1-B5) has a higher affinity to Ig (1×10^{10} M^{-1}), four of these domains have been expressed together in the following way:

PL-N and PL-C2 are synthetic oligonucleotides (manufactured at the Biomolecular Unit at Lund University (Sweden) in accordance with Applicant's instructions) which were used, with the aid of PCR (Polymerase Chain Reaction) to amplify a clonable gene fragment, called B1-4, which codes for four Ig-binding protein L domains (ala-val-glu-asn-B1-B2-B3-B4-lys-lys-val-asp-glu-lys-pro-glu-glu):

PL-N: 5'-GCTCAGGCGGCGCCGGTAGAAAATAAAGAAGAAACACCAGAAAC-3'
valgluasnlysglugluthrproglu

Pl-C2: 5'-CAGCAGCAGCCATGGGTTCTTCTGGTTTTTCGTCAACTTTCTTA-
3'

Amino acids have been shown under corresponding triplets in the coded strand. DNA-fragments which have been amplified with the aid of PL-N contain the recognition sequence for the restriction enzyme **HpaII** immediately upstream of the triplet which codes for the first amino acid (val) in the expressed protein L-fragment. The fragment that has been cleaved with **HpaII** can be ligated with DNA (in this case, the used expression vector pHD389) which has been cleaved with **NarI**. The construction results in translation of an extra amino acid (ala) immediately upstream of the first amino acid in the protein L-fragment. The DNA-fragment that has been amplified with the aid of PL-C2 will contain the recognition sequence for the restriction enzyme **NcoI** (emphasized) immediately downstream of the sequence which codes for the last amino acid in the expressed protein L-fragment (glu). Amplified fragments which have been cleaved with **NcoI** can be ligated to the NcoI-cleaved, PCR-generated protein-asp-CDC-met-fragment (see below).

Protein G

It is known that a simple C-domain from protein G will bind to IgG (B. Guss, M. Eliasson, A. Olsson, M. Uhlen, A.-K. Frej, H. Jörnvall, I. Flock and M. Lindberg. 1986. Structure of the IgG-binding regions of streptococcal protein G. EMBO. J. 5: 1567-1575). The strength at which a simple C-domain binds to IgG is relatively low (5×10^{7} M^{-1}). A fragment which consists of two C-domains with an intermediate D-region having a length of 15 amino acids, however, has a considerably higher affinity to IgG (1×10^{9} M^{-1}). CDC-N and CDC-C are oligonucleotides which have been used as PCR-primers to amplify a clonable DNA-fragment, designated CDC, which

codes for two IgG-binding protein G-domains (pro-met-asp-CDC-met).

CDC-N: GGCCATGGACACTTACAAATTAATCCTTAATGGT
metaspthrtyrlysleuileleuasngly

CDC-C: CAGGTCGACTTATTACATTTCAGTTACCGTAAAGGTCTTAGT

Amino acids in the resultant sequence have been shown beneath the primer of the coding strand. DNA-fragments which have been amplified with the aid of CDC-N contain the recognition sequence for the restriction enzyme **NcoI** (marked with a line above the sequence). Cleaved amplified fragments can be ligated with the fragment that has been amplified with the aid of PL-C2 and then cleaved with **NcoI**. The fragment will therewith be translated to the correct reading frame. DNA-fragments which have been amplified with the aid of CDC-C will contain two so-called stop condons (emphasized) which terminate translation. The recognition sequence for the restriction enzyme **SalI** (marked with a line above the sequence) follows immediately afterwards, this sequence also being found in the expression vector pHD389 (see Figure 1).

Those sequences which code for the binding properties of protein L (B1-B5) and for protein G (CDC) respectively contain no internal recognition sequences for the restriction enzymes **HpaII, SalI** or **NcoI**.

Amplification and cloning procedures

PCR (Polymerase Chain Reaction) was carried out in accordance with a protocol described by Saiki et al., 1988; PCR was carried out in a Hybaid Intelligent Heating-block (Teddington, UK): 100 µl of the reaction mixture contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5

mM $MgCl_2$, 100 µg/ml gelatine, 300 µM with respect to each of the deoxynucleotides (dATP, dCTP, dGTP, dTTP), (Pharmacia). In order to amplify sequences which code for the light-chain binding parts of protein L, there were added 20 pmol of each of the oligonucleotides PL-N and PL-C2, and 10 µl of a DNA-solution which contained 0.1 mg/ml of chromosomal DNA from Peptostreptococcus magnus, strain 312. By way of an alternative, 20 pmol were added to each of the oligonucleotide pairs CDC-N and CDC-C and 10 µl of a DNA-solution which contained 0.1 mg/ml of chromosomal DNA from a group C streptococcus strain (Streptococcus equisimilis) called C40 (U. Sjöbring, L. Björck and W. Kastern. 1991. Streptococcal protein G: Gene structure and protein binding properties. J. Biol. Chem. 266: 399-405 or with **NcoI** and **SalI** (10 U/µg PCR-product), (for CDC) at 37°C. The thus amplified and subseqently cleaved DNA-fragments were then separated by electrophoresis in a 2% (weight by volume) agrose gel (NuSieve agarose, FMC Bioproducts) in a TAE-buffer (40 mM Tris, 20 mM aNa-cetate, 2 mM EDTA, pH 8.0). The resultant fragments, 930 bp (for B1-4) and 390 bp (for CDC) were cut from the gel. The concentration of DNA in the thus separated gel pieces was estimated to be 0.05 mg/ml. The agarose pieces cut from the gel and containing the cleaved, amplified fragments (B1-4 and CDC) were melted in a water bath at 65°C, whereafter they were allowed to cool to 37°C. 10 µl (0.5 µg) of this DNA were transferred to a semi-microtube (Sarstedt), preheated to 37°C, whereafter 1 µl of the vector pHD389 which had been cleaved with **NarI** and **SalI** were added. 1 µl 10 x ligase buffer (Promega) and 1 µl T4 DNA-ligase (1 unit/µl) were also added. The ligating reaction was permitted to take place at 37°C for 6 hours. The cleaving and ligating conditions recommended by the producer of DNA-ligase and restriction enzymes (Promega) were followed in other respects. The

ligating reaction was then used to transform E. coli, strain LE392, which had been made competent in accordance with the rubidium-chloride/calcium-dichloride method as described by Kushner (1978). Manipulation of DNA was effected in accordance with molecular biological standard methods (Sambrook et al., 1989).

Expression system

The vector pHD389 (see Figure 2) is a modified variant of the plasmid pHD313 (Dalböge et al., 1989). The vector which was replicated in E. coli (contains origin of replication from plasmid pUC19) is constructed such that DNA-fragments which have been cloned in the cleaving site for **NarI** will be expressed immediately after, or downstream, of the signal peptide (21 amino acids) from the envelope protein **ompA** from E. coli. Translation will be initiated from the ATG-codon which codes for the first amino acid (methicnine) in the signal peptide. The construction with an E. coli-individual signal sequence which precedes the desired peptide enables the translated peptide to be transported to the periplasmic space in E. coli. This is beneficial since it reduces the risk of degradation of the desired product through the intracellular occurrent enzymes of E. coli. Furthermore, it is easier to purify peptides which have been exported to the periplasmatic space. Unique recognition sequences (multiple cloning sequences) for several other restriction enzymes, among them **EcoRI**, **SalI** and **BamHI** are present immediately downstream of the **NarI** cleaving site. An optimized so-called Shine-Dalgarno sequence (also called ribosomal binding site, RBS) is found seven nucleotides upstream of the ATG-codon in the signal sequence from **ompA**, this optimized Shbine-Dalgarno sequence binding to a complementary sequence in 16S rRNA in the ribosomes and in a manner to decide that the

translation is initiated in the correct place. The transcription of such DNA as that which is co-transcribed with the signal sequence for ompA is controlled by the P_R-promotor from coliphage λ. The vector also contains the gene for cI857 from coliphage λ, the product of which regulates-down transcription from P_R and the product of which is expressed constitutively. This cI857-mediated down-regulation of transcription from P_R is heat-sensitive. Transcription which is regulated, or controlled, from this promotor will be terminated with the aid of a so-called rho-independent transcription terminating sequence which is inserted in the vector immediately downstream of the multiple cloning site. The plasmid also carries the gene for ß-lactamase (from the plasmid pUC19), the product of which permits ampicillin-selection of E. coli clones that have been transformed with the vector.

Selection of protein LG-produced clones

The transformed bacteria are cultivated on culture plates with LB-medium which also contained ampicillin in a concentration of 100 µg/ml. The bacteria were cultivated overnight at 30°C, whereafter they were transferred to a cultivation cabinet (42°C) and cultured for a further four (4) hours. The plates were stored in a refrigerator overnight. On the following day, the colonies were transferred to nitrocellulose filters. The filters and culture plates were marked, so that the transferred colonies could later be identified on the culture plate. The culture plates were again incubated overnight at 30°C, so that rests of transferred bacteria colonies remaining on the plates could again grow. The plates were then stored in a refrigerator. The filter was incubated in 10% SDS for 10 minutes, so as to lyse the bacteria in the colonies on the nitrocellulose

impression. Filters containing lysed bacteria were then rinsed with a blocking buffer consisting of PBS (pH 7.2) with 0.25% gelatine and 0.25% Tween-20 (four baths of 250 ml at 37°C), whereafter the filter was incubated with radioactively (marked with ^{125}I according to the chloromine-T-method) marked Ig-κ-chains (20 ng/ml) in PBS with 0.1% gelatine). The incubation process took place at room temperature for four (4) hours, whereafter non-bound radioactively marked protein was rinsed-off with PBS (pH 7.2) containing 0.5 M NaCl, 0.25% gelatine and 0.25% Tween-20 (four baths, 250 ml each at room temperature). All filters were exposed to X-ray film. Positive colonies on the original culture plate were identified. A number of positive colonies were re-cultivated on new plates and new colony-blot experiments were carried out with these plates as a starting material with the intention of identifying E. coli colonies which bind IgG Fc. These tests were carried out in precisely the same manner as that described above with respect to the identification of E. coli-colonies which expressed Ig light-chain-binding protein, with the exception that a radioactively marked (^{125}I) IgG Fc (20 ng/ml) was used as a probe. Clones which reacted with both proteins were selected and analyzed with regard to the size of the DNA-fragment introduced in the vector. One of these clones was chosen for production of protein LG, pHDLG. The DNA't taken from this clone and introduced into plasmid pHD389 was sequenced. The DNA-sequence exhibited full agreement with corresponding sequences (B1-B4 and 21 bases in B5) in the gene for protein L from Peptostreptococcus magnus, strain 312, and with C1DC2 sequence in group C streptococcus strain C40. The size and binding properties of the protein produced from clone pHDLG was analyzed with the aid of SDS-PAGE (see Figure 8), dot-blot experiment (see Figure 10) and competitive binding experiments.

Production of protein LG

Several colonies from a culture plate with E. coli pHDLG were used to inoculate a preculture (LB-medium with an addition of 100 mg/l ampicillin) were cultivated at 28°C overnight. In the morning, the preculture was transferred to a larger volume (100 times the volume of the preculture) of fresh LB-medium containing ampicillin (100 mg/l) and was cultivated in vibrating flasks (200 rpm), (or fermenters) at 28°C. When an absorbence value of 0.5 was reached at 620 nm, the cultivation temperature was raised to 40°C (induction of transcription). The cultivation process was then continued for 4 hours (applies only to cultivation in vibrated flasks). The bacteria were centrifuged down upon termination of the cultivation process. The bacteria were then lysed at 4°C in accordance with an osmotic shock method (Dalböge et al., 1989). The lysate was adjusted to a pH of 7. Remaining bacteria rests were centrifuged down and the supernatent then purified on IgG-sepharose, in accordance with the protocol earlier described with reference to protein G and protein L. (Sjöbring et al., 1991, Kastern et al., 1992).

The expression system gave about 30 mg/l of protein LG when cultivation in vibrated flasks. A deposition has been made at DSSM, Identification Reference DSSM E. coli LE392/pHDLG.

Example 3

Analysis of the binding properties of protein LG

Western Blot

Protein G (the C1DC2-fragment), protein L (four B-

domains) and protein LG were isolated with SDS-PAGE (10% acrylamide concentration). The isolated proteins were transfered to nitrocellulose membranes in three similar copies (triplicate). Each of these membranes was incubated with radioactively marked proteins (20 ng/ml: one of the membrane-copies was incubated with human polyclonal IgG, another with human IgG Fc-fragment and the third with isolated human IgG χchains. Non-bound radioactively marked proteins were rinsed off and all filters were then exposed to X-ray film.

Slot-blot

Human polyclonal Ig-preparations and Ig-fragments were applied with the aid of a slot-blot appliances on nitrocellulose filters in given quantities (see Figure 10) on three similar copies. Each of these membranes was incubated with radioactively marked proteins (20 ng/ml). One of the membrane copies was incubated with protein LG, another with protein L and the third with protein G. Non-bound radioactively marked proteins were rinsed-off and all filters were then exposed to X-ray film.

The results are shown in Figures 9 and 10.

Other binding experiments have been carried out, with the following results:

TABLE

Binding of the proteins G, L and LG to immunoglobulins.

Binding protein:	G	K_a	L	K_a	LG	K_a
Immunoglobulin						
Human:						
Polyclonal IgG*	+	67 (10)	+	9.0	+	20
IgG subclasses						
IgG_1	+	2.0	+		+	
IgG_2	+	3.1	+		+	
IgG_3	+	6.1	+		+	
IgG_4	+	4.7	+		+	
IgG fragment						
Fc*	+	6.0 (0.5)	-			+
$F(ab')_2$*	+	0.4 (0.2)	+		+	
kappa	-		+	1.5	+	
lambda	-		(-)#			
Other Ig-classes						
IgM	-		+	11.6	+	
IgA	-		+	10.4	+	
IgE	-		+		+	
IgD	-					
Other Species:						
Polyclonal						
Monkey	+		+		+	
Rabbit IgG	+	70	+	0.074	+	
IgG-Fc	+	3.0	-		+	
$IgG\text{-}F(ab')_2$	+	0.44			+	
Mouse	+	41	+	2.6	+	
Rat	+	1.5	+	0.39	+	
Goat	+	14	-		+	

TABLE (cont'd.)

Binding of the proteins G, L and LG to immunoglobulins.

Binding protein:		G	K_a	L	K_a	LG	K_a
Immunoglobulin							
Bovine	IgG_1	+	3	-		+	
	IgG_2	+	2	-		+	
Horse		+		-		+	
Guinea Pig		+		+		+	
Sheep		+		-		+	
Dog		+		-		+	
Pig		+		+		+	
Hamster		+					
Cat		-		-			
Hen		-		-			
Monclonals[&]							
Mouse							
IgG_1		+		+		+	
IgG_{2a}		+		+		+	
IgG_{2b}		+				+	
IgG_3		+				+	
IgM		-		+		+	
IgA		-		+		+	
Rat							
IgG_{2a}		+		+		+	
IgG_{2b}		+				+	
IgG_{2c}		+				+	

K_a = affinity constant (M^{-1}). *The numerals within parenthesis disclose the affinity of a recombinant protein G comprised of two IgG-binding domains. #A weak bond to lambda chains exists. &Binding to Pl and PLG depends on the type of light chain of Ig.

It will thus be seen that the synthesized hybrid protein LG has a broad binding activity/specificity.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: HighTech Receptro AB
(B) STREET: c/o Active, Skeppsbron 2
(C) CITY: MALMO
(E) COUNTRY: SWEDEN
(F) POSTAL CODE (ZIP): 211 20
(G) TELEPHONE: 040/35 07 00
(H) TELEFAX: 040/ 23 74 05
(I) TELEX: 32637 Active S

(ii) TITLE OF INVENTION: Hybridprotein

(iii) NUMBER OF SEQUENCES: 1

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
(EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: SE PCT/SE93/00375
(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: SE 9201331-7
(B) FILING DATE: 28-APR-1992

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 305 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli LE392/pHDL, DSM 7054

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Ala Val Glu Asn Lys Glu Glu Thr Pro Glu Thr Pro Glu Thr Asp Ser
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Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu
        35                  40                  45
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Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
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Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
85 90 95

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
100 105 110

Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu
115 120 125

Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr
130 135 140

Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro
145 150 155 160

Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys
165 170 175

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu
180 185 190

Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr
195 200 205

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
210 215 220

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
225 230 235 240

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
245 250 255

Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu
260 265 270

Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr
275 280 285

Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu
290 295 300

Glu
305

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 921 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli LE392/pHDL, DSM 7054

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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AGAAGAAGTA ACAATCAAAG CTAACCTAAT CTTTGCAAAT GGAAGCACAC 100

AAACTGCAGA ATTCAAAGGA ACATTTGAAA AAGCAACATC AGAAGCTTAT 150

GCGTATGCAG ATACTTTGAA GAAAGACAAT GGAGAATATA CTGTAGATGT 200

TGCAGATAAA GGTTATACTT TAAATATTAA ATTTGCTGGA AAAGAAAAAA 250

CACCAGAAGA ACCAAAAGAA GAAGTTACTA TTAAAGCAAA CTTAATCTAT 300

GCAGATGGAA AAACACAAAC AGCAGAATTC AAAGGAACAT TTGAAGAAGC 350

AACAGCAGAA GCATACAGAT ATGCAGATGC ATTAAAGAAG GACAATGGAG 400

AATATACAGT AGACGTTGCA GATAAAGGTT ATACTTTAAA TATTAAATTT 450

GCTGGAAAAG AAAAAACACC AGAAGAACCA AAAGAAGAAG TTACTATTAA 500

AGCAAACTTA ATCTATGCAG ATGGAAAAAC ACAAACAGCA GAATTCAAAG 550

GAACATTTGA AGAAGCAACA GCAGAAGCAT ACAGATATGC TGACTTATTA 600

GCAAAAGAAA ATGGTAAATA TACAGTAGAC GTTGCAGATA AAGGTTATAC 650

TTTAAATATT AAATTTGCTG GAAAAGAAAA AACACCAGAA GAACCAAAAG 700

AAGAAGTTAC TATTAAAGCA AACTTAATCT ATGCAGATGG AAAAACTCAA 750

ACAGCAGAGT TCAAAGGAAC ATTTGCAGAA GCAACAGCAG AAGCATACAG 800

ATACGCTGAC TTATTAGCAA AAGAAAATGG TAAATATACA GCAGACTTAG 850

AAGATGGTGG ATACACTATT AATATTAGAT TTGCAGGTAA GAAAGTTGAC 900

GAAAAACCAG AAGAATAATA A 921

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 434 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli LE392/pHDLG, DSM 7055

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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Glu Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Phe Ala Asn Gly Ser
            20                  25                  30

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu
        35                  40                  45

Ala Tyr Ala Tyr Ala Asp Thr Leu Lys Lys Asp Asn Gly Glu Tyr Thr
    50                  55                  60

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
65                  70                  75                  80

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
                85                  90                  95

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
            100                 105                 110

Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu
        115                 120                 125

Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr
    130                 135                 140

Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro
145                 150                 155                 160

Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys
                165                 170                 175

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu
            180                 185                 190

Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr
        195                 200                 205

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
    210                 215                 220

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
225                 230                 235                 240

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
                245                 250                 255

Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu
            260                 265                 270
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Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr
275 280 285

Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu
290 295 300

Glu Pro Met Asp Thr Tyr Lys Leu Ile Leu Asn Gly Lys Thr Leu Lys
305 310 315 320

Gly Glu Thr Thr Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val
325 330 335

Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr
340 345 350

Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Glu Val Ile
355 360 365

Asp Ala Ser Glu Leu Thr Pro Ala Val Thr Thr Tyr Lys Leu Val Ile
370 375 380

Asn Gly Lys Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Ala
385 390 395 400

Glu Thr Ala Glu Lys Ala Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val
405 410 415

Asp Gly Val Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr
420 425 430

Glu Met

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1308 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichis coli L392/pHDLG, DSM 7055

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GCGGTAGAAA ATAAAGAAGA AACACCAGAA ACACCAGAAA CTGATTCAGA 50

AGAAGAAGTA ACAATCAAAG CTAACCTAAT CTTTGCAAAT GGAAGCACAC 100

AAACTGCAGA ATTCAAAGGA ACATTTGAAA AAGCAACATC AGAAGCTTAT 150

GCGTATGCAG ATACTTTGAA GAAAGACAAT GGAGAATATA CTGTAGATGT 200

TGCAGATAAA GGTTATACTT TAAATATTAA ATTTGCTGGA AAAGAAAAAA 250

CACCAGAAGA ACCAAAAGAA GAAGTTACTA TTAAAGCAAA CTTAATCTAT 300

GCAGATGGAA AAACACAAAC AGCAGAATTC AAAGGAACAT TTGAAGAAGC 350

AACAGCAGAA GCATACAGAT ATGCAGATGC ATTAAAGAAG GACAATGGAG 400

AATATACAGT AGACGTTGCA GATAAAGGTT ATACTTTAAA TATTAAATTT 450

GCTGGAAAAG AAAAAACACC AGAAGAACCA AAAGAAGAAG TTACTATTAA 500

AGCAAACTTA ATCTATGCAG ATGGAAAAAC ACAAACAGCA GAATTCAAAG 550

GAACATTTGA AGAAGCAACA GCAGAAGCAT ACAGATATGC TGACTTATTA 600

GCAAAAGAAA ATGGTAAATA TACAGTAGAC GTTGCAGATA AAGGTTATAC 650

TTTAAATATT AAATTTGCTG GAAAAGAAAA AACACCAGAA GAACCAAAAG 700

AAGAAGTTAC TATTAAAGCA AACTTAATCT ATGCAGATGG AAAAACTCAA 750

ACAGCAGAGT TCAAAGGAAC ATTTGCAGAA GCAACAGCAG AAGCATACAG 800

ATACGCTGAC TTATTAGCAA AAGAAAATGG TAAATATACA GCAGACTTAG 850

AAGATGGTGG ATACACTATT AATATTAGAT TTGCAGGTAA GAAAGTTGAC 900

GAAAAACCAG AAGAACCCAT GGACACTTAC AAATTAATCC TTAATGGTAA 950

AACATTGAAA GGCGAAACAA CTACTGAAGC TGTTGATGCT GCTACTGCAG 1000

AAAAAGTCTT CAAACAATAC GCTAACGACA ACGGTGTTGA CGGTGAATGG 1050

ACTTACGACG ATGCGACTAA GACCTTTACA GTTACTGAAA AACCAGAAGT 1100

GATCGATGCG TCTGAATTAA CACCAGCCGT GACAACTTAC AAACTTGTTA 1150

TTAATGGTAA AACATTGAAA GGCGAAACAA CTACTAAAGC AGTAGACGCA 1200

GAAACTGCAG AAAAAGCCTT CAAACAATAC GCTAACGACA ACGGTGTTGA 1250

TGGTGTTTGG ACTTATGATG ATGCGACTAA GACCTTTACG GTAACTGAAA 1300

TGTAATAA 1308

Claims

1/ Protein L having the ability to bind to the light chains of immunoglobulins, c h a r a c t e r i z e d in that the protein L has the following amino acid sequence:

— B1

Ala Val Glu Asn Lys Glu Glu Thr Pro Glu Thr Pro Glu Thr Asp Ser
1 5 10 15

Glu Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Phe Ala Asn Gly Ser
20 25 30

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu
35 40 45

Ala Tyr Ala Tyr Ala Asp Thr Leu Lys Lys Asp Asn Gly Glu Tyr Thr
50 55 60

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
65 B2 70 75 80

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
85 90 95

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
100 105 110

Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu
115 120 125

Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr
130 135 140

— B3

Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro
145 150 155 160

Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys
165 170 175

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu
180 185 190

Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr
195 200 205

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
210 215 220

—— B4

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
225 230 235 240

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
245 250 255

Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu
260 265 270

Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr
275 280 — B5 285

Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu
290 295 300

Glu

and variants, subfragments, multiples or mixtures of the domains B1-B5 having the same binding properties.

2. DNA-sequence, characterized in that it codes for the protein according to Claim 1 and has the following nucleotide sequence:

GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA -8
GAA GAA GAA GTA ACA ATC AAA GCT AAC CTA ATC TTT GCA AAT GGA AGC 36
ACA CAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA 144
GCT TAT GCG TAT GCA GAT ACT TTG AAG AAA GAC AAT GGA GAA TAT ACT 192
GTA GAT GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 240
AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 288
AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA 336
ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA 384
AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT -32
ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA 480
AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA 528

ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA 576

GCA TAC AGA TAT GCT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA 624

GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 672

AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 720

AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA 768

ACA TTT GCA GAA GCA ACA GCA GAA GCA TAC AGA TAC GCT GAC TTA TTA 816

GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC 864

ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA 912

GAA TAATAA 927

3. A hybrid protein, characterized in that it includes one or more of the B1-B5-domains according to Claim 1 which bind to the light chains in immunoglobulins of all classes, and domains which bind to heavy chains in immunoglobulin G.

4. A hybrid protein according to Claim 3, characterized in that the domains which bind to heavy chains in immunoglobulin G are chosen from among the C1- and C2-domains in protein G or from among any other functionally similar proteins which bind to heavy chains in immunoglobulin G, and variants, subfragments, multiples or mixtures thereof having the same binding properties.

5. A hybrid protein according to Claim 4, characterized in that the hybrid protein has the following amino acid sequence:

Ala Val Glu Asn Lys Glu Glu Thr Pro Glu Thr Pro Glu Thr Asp Ser
1 5 10 15

Glu Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Phe Ala Asn Gly Ser
20 25 30

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu
35 40 45

Ala Tyr Ala Tyr Ala Asp Thr Leu Lys Lys Asp Asn Gly Glu Tyr Thr
50 55 60

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
65 70 75 80

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
85 90 95

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
100 105 110

Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu
115 120 125

Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr
130 135 140

Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro
145 150 155 160

Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys
165 170 175

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu
180 185 190

Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr
195 200 205

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
210 215 220

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
225 230 235 240

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
245 250 255

Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu
260 265 270

Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr
275 280 285

Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu
290 295 300

Glu Pro Met Asp Thr Tyr Lys Leu Ile Leu Asn Gly Lys Thr Leu Lys
305 310 315 320

Gly Glu Thr Thr Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val
325 330 335

Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr
340 345 350

Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Glu Val Ile
355 360 365

Asp Ala Ser Glu Leu Thr Pro Ala Val Thr Thr Tyr Lys Leu Val Ile
370 375 380

Asn Gly Lys Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Ala
385 390 395 400

Glu Thr Ala Glu Lys Ala Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val
405 410 415

Asp Gly Val Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr
420 425 430

Glu Met

and variants, subfragments, multiples or mixtures of the domains B1-B5 having the same binding properties.

6. DNA-sequence, characterized in that it codes for a protein according to Claim 5 and has the following nucleotide sequence:

GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA 48

GAA GAA GAA GTA ACA ATC AAA GCT AAC CTA ATC TTT GCA AAT GGA AGC 96

ACA GAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA 144

GCT TAT GCG TAT GCA GAT ACT TTG AAG AAA GAC AAT GGA GAA TAT ACT 192

GTA GAT GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 240

AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 288

AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA 336

ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA 384

AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT 432

ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA 480

AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA 528

ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA 576

GCA TAC AGA TAT GCT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA 624

GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 672

AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 720

AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA 768

ACA TTT GCA GAA GCA ACA GCA GAA GCA TAC AGA TAC GCT GAC TTA TTA 816

GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC 864

ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA 912

GAA CCC ATG GAC ACT TAC AAA TTA ATC CTT AAT GGT AAA ACA TTG AAA 960

GGC GAA ACA ACT ACT GAA GCT GTT GAT GCT GCT ACT GCA GAA AAA GTC 1008

TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT GAC GGT GAA TGG ACT TAC 1056

GAC GAT GCG ACT AAG ACC TTT ACA GTT ACT GAA AAA CCA GAA GTG ATC 1104

GAT GCG TCT GAA TCA ACA CCA GCC GTG ACA ACT TAC AAA CTT GTT ATT 1152

AAT GGT AAA ACA TTG AAA GGC GAA ACA ACT ACT AAA GCA GTA GAC GCA 1200

GAA ACT GCA GAA AAA GCC TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT 1248

GAT GGT GTT TGG ACT TAT GAT GAT GCG ACT AAG ACC TTT ACG GTA ACT 1296

GAA ATG TAATAA 1308

7. DNA-sequence, c h a r a c t e r i z e d in that it codes for a protein according to Claims 3, 4 and 5.

8. A plasmid vector, c h a r a c t e r i z e d in that it includes a DNA-sequence according to any one of Claims 2 and 6-8, preferably the vector pHDLG or pHDL according to Fig. 3 or 4.

9. A host cell, c h a r a c t e r i z e d in that it is transformed with the hybrid plasmid according to Claim 9, in particular a host which belongs to the species E. coli, particularly E. coli LE392, or Bacillus subtilis, Saccaromyces cerevisiae, preferably Id. Ref. DSSM E. coli LE392 pHDL and E. coli LE392/pHDLG respectively.

10. A method for producing a protein according to Claims 1 and 3-5, c h a r a c t e r i z e d by cultivating a host cell according to Claim 10 under suitable conditions; accumulating the protein in the culture or lysing the cells and extracting the protein therefrom.

11. A reagent kit for binding, separating and identifying immunoglobulins, c h a r a c t e r i z e d in that it includes a protein according to any one of Claims 1 and 3-5.

12. A composition, c h a r a c t e r i z e d in that it includes a protein according to any one of Claims 1 and 3-5, and optionally additives or carriers.

13. A pharmaceutical composition, c h a r a c t e r i z e d in that it includes a protein according to any one of Claims 1 and 3-5, and optionally a pharmaceutically acceptable carrier or extender.

ABSTRACT OF THE DISCLOSURE

The invention relates to sequences of protein L which bind to light chains of immunoglobulins. The invention also relates to hybrid proteins thereof which are able to bind to both light and heavy chains of immunoglobulin G, in particular protein LG. The invention also relates to DNA-sequences which code for the proteins, vectors which include such DNA-sequences, host cells which have been transformed with the vectors, methods for producing the proteins, reagent appliances for separation and identification of immunoglobulins, compositions and pharmaceutical compositions and pharmaceutical compositions which contain the proteins.

FOR UTILITY/DESIGN
CIP/PLANT
ORIGINAL/SUBSTITUTE
DECLARATIONS

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

CUSHMAN
FORM

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROTEIN L AND HYBRID PROTEINS THEREOF

the specification of which (CHECK applicable BOX(ES))

X
X(ES)

→ ☐ is attached hereto.

→ ☐ was filed on. ... as U.S. Application Serial No. ...

→ ☐ was filed as PCT international Application No. PCT/ SE93 / 00375 on April 28, 1993

ar d (if applicable to U S or PCT application) was amended on ...

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above, to the best of my ability I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 C.F.R. 1.56(a) and 35 U S C. 102 and 103, each as set forth on the reverse side hereof. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing date of this application

PRIOR FOREIGN APPLICATION(S)			PRIORITY CLAIMED	
Number	Country	Day/MONTH/Year Filed	Yes	No
9201331-7		28-04-1992	X	

I hereby claim the benefit under 35 U S C 120/365 of all United States applications listed below and PCT international applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U S C 112, I acknowledge the duty to disclose material information as defined in 37 C F R 1 56(a) which occurred between the filing date of the prior applications and the national or PCT international filing date of this application

PRIOR U S OR PCT APPLICATIONS		Status patented,
Application Serial No	Day/MONTH/Year Filed	pending, abandoned
PCT/SE93/00375	28-04-1993	

I hereby declare that all statements made herein of my own knowledge are true and that all sta ents made on formation and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may je opardize the validity of the application or any patent issued thereon

And I hereby appoint Cushman Darby & Cushman, Eleventh Floor, 1615 L Street, N W Washing n, D C 20036-5601, telephone number 861-3000 (to whom all communications are to be directed) and the below named partners thereof (of the same ad ress) individually and c llectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Cushman, Darby & Cushman in writing to the contrary.

Paul N Kokulis	16773	Lawrence A Hymo	19057	George M Sirilla	18221	Watson T Scott,	26581
Allen Kirkpatrick	16749	Edgar H Martin	20534	William T Bullinger	25503	Peter W Gowdey	25872
George T Mobile	17353	William K West, Jr	22057	Donald J Bird	25323	Dale S Lazar	28872
James L Dooley	17710	Kevin E Joyce	20508	James R Longacre	24421	Glenn J Perry	28458
Raymond F Lippitt	17519	Edward M Prince	22429	W Warren Taltavull	25647	Kendrew H Colton	30368
G Lloyd Knight	17698	Donald B Deaver	23048	Michael L Keller	26751	Chris Comuntzis	31097
Carl G Love	18781	David W Brinkman	20817	Charles R Donohoe	24546		

1) INVENTOR'S SIGNATURE [signature] Date 23 September 1994

Inventor's Name (typed) Lars Björck Swedish
First Middle Initial Family Name Citizenship

Residence (City) Södra Sandby (State/Foreign Country) Sweden

Post Office Address (Include Zip Code) Kornvägen 40, S-240 17 Södra Sandby

2) INVENTOR'S SIGNATURE [signature] Date 23 September 1994

Inventor's Name (typed) Ulf Sjöbring Swedish
First Middle Initial Family Name Citizenship

Residence (City) Lund (State/Foreign Country) Sweden

Post Office Address (Include Zip Code) Lilla Sigridsgatan 1, S-223 50 Lund

3) INVENTOR'S SIGNATURE Date

Inventor's Name (typed)
First Middle Initial Family Name Citizenship

Residence (City) (State/Foreign Country)

Post Office Address (Include Zip Code)

4) INVENTOR'S SIGNATURE Date

Inventor's Name (typed)
First Middle Initial Family Name Citizenship

Residence (City) (State/Foreign Country)

Post Office Address (Include Zip Code)

FOR ADDITIONAL INVENTORS, check box ☐ and attach sheet with same information and signature and date for each.

CDC-116 5/88

(over)

NOTE: DO NOT copy this form without also copying reverse side too for inventors.

UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee: Lars BJÖRCK et al. Attorney's Docket No.: DWB 216764
Serial or Patent No.:
Filed or Issued: October 26, 1994
For: PROTEIN L AND HYBRID PROTEINS THEREOF

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9 (f) and 1.27 (b)) — INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9 (c) for purposes of paying reduced fees under section 41 (a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled PROTEIN L AND HYBRID PROTEINS THEREOF described in

[X] the specification filed herewith
[ ] application serial no. _____, filed October 26, 1994.
[ ] patent no. _____, issued _____.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9 (c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[ ] no such person, concern, or organization
[X] persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME HighTech Receptor AB
ADDRESS c/o Active i Malmö AB, Stora Nygatan 61, S-211 37 Malmö
[ ] INDIVIDUAL [X] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Lars Björck		Ulf Sjöbring
NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
Lars Björck		Ulf Sjöbring
Signature of Inventor	Signature of Inventor	Signature of Inventor
September 23 1994		September 23 1994
Date	Date	Date

Form PTO-FB-A410 (8-83)

(January 1984)

Applicant or Patentee: Lars BJÖRCK et al. Attorney's DWB
Serial or Patent No.: Docket No.: M.216764
Filed or Issued: October 26, 1994
For: PROTEIN L AND HYBRID PROTEINS THEREOF

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9 (f) and 1.27 (c)) — SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below:
[X] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN HighTech Receptor AB
ADDRESS OF CONCERN c/o Active i Malmö AB, Stora Nygatan 61
S-211 37 Malmö, Sweden

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9 (d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full time, part time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled PROTEIN L AND HYBRID PROTEINS THEREOF by inventor(s) Lars BJÖRCK et al. described in

[X] the specification filed herewith
[ ] application serial no. , filed October 26, 1994
[ ] patent no. , issued

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME
ADDRESS
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING ① JONAS LIND ② KARL-G. BRANTÉN
TITLE OF PERSON OTHER THAN OWNER ① CHAIRMAN ② PROJECT MANAGER
ADDRESS OF PERSON SIGNING ① SE-"NAME OF LONDON" ② SEE "NAME OF COMPANY"

SIGNATURE ① ② DATE 22 SEPT, 1994

Form PTO-FD-A430 (8-83) (January 1981)

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3406
|
AAGCTTAAGGAGGTTAATCG ATG AAA AAA ACT GCT ATC GCT ATC GCT GTT
H  A          C    met
i  f  RBS     l
n  l          a
3  2          1
GCT CTG GCT GGT TTC GCT ACT GTT GCT CAG GCG GCG CCG AGA TCT
                                    ala N      B
                                        a      g
                                        r      I
                                        1      2
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    E      S      K   X      B      X      S
    c      a      p   m      a      b      a
    o      c      h   a      m      a      l
    I      1      1   1      1      1      1

CTG CAG GCA TGC
P      S     |
s      p     |
t      h     3557
1      1
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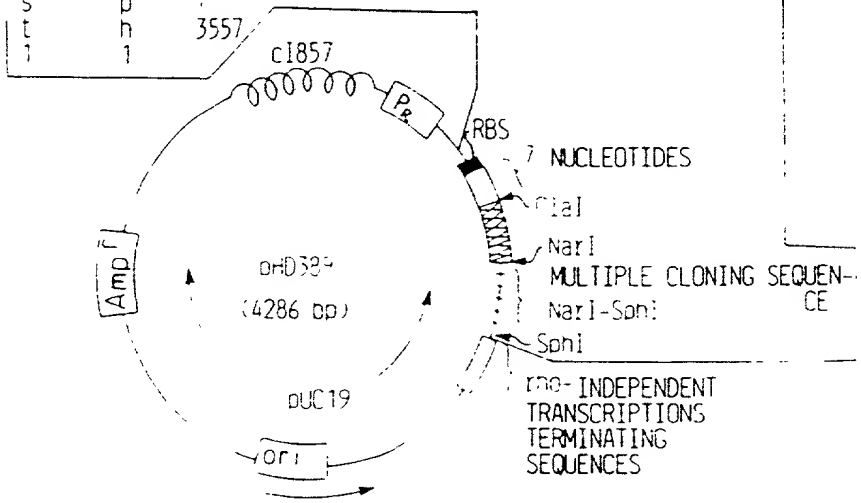


▨ SIGNAL PEPTIDE FOR THE SEQUENCE FROM OmpA

■

RBS = RIBOSOMAL BINDING SEQUENCE AGGAGG

P_R = "RIGHT" PROMOTOR FROM COLIPHAGE λ

cI857 THE GENE FOR A HEAT-SENEITIVE REPRESSOR-PROTEIN FROM COLIPHAGE λ

MULTIPLE CLONING SEQUENCE

UNIQUE CLEAVING SITES: NarI, BglII, EcoRI, SacI, KpnI, XmaI, BamHI, XbaI, SalI, PstI, SphI

FIG. 1 PLASMA pHD 389. THE RIBOSOMAL BINDING-SEQUENCE (EMPHASIZED WITH A FULL LINE). THE SEQUENCE FOR SIGNAL PEPTIDE FROM ompA (FROM E.coli) (DOTTED LINE) AND RECOGNITION SEQUENCE FOR SEVERAL RESTRICTION ENZYMES ARE SHOWN.

08/325218

PROTEIN LG

FIG.2 (CONT.)

FIG.2
(CONT.)

SUBSTITUTE SHEET

```
GCAACAGCAGAAGCATACAGATACGCTGACTTATTAGCAAAAGAAAATGGTAAATATACA   840
AlaThrAlaGluAlaTyrArgTyrAlaAspLeuLeuAlaLysGluAsnGlyLysTyrThr   280
                                                  B5
GCAGACTTAGAAGATGGTGGATACACTATTAATATTAGATTTGCAGGTAAGAAAGTTGAC   900
AlaAspLeuGluAspGlyGlyTyrThrIleAsnIleArgPheAlaGlyLysLysValAsp   300
                       C1
GAAAAACCAGAAGAACCCATGGACACTTACAAATTAATCCTTAATGGTAAAACATTGAAA   960
GluLysProGluGluProMetAspThrTyrLysLeuIleLeuAsnGlyLysThrLeuLys   320

GGCGAAACAACTACTGAAGCTGTTGATGCTGCTACTGCAGAAAAAGTCTTCAAACAATAC  1020
GlyGluThrThrThrGluAlaValAspAlaAlaThrAlaGluLysValPheLysGlnTyr   340

GCTAACGACAACGGTGTTGACGGTGAATGGACTTACGACGATGCGACTAAGACCTTTACA  1080
AlaAsnAspAsnGlyValAspGlyGluTrpThrTyrAspAspAlaThrLysThrPheThr   360
         D                                              C2
GTTACTGAAAAACCAGAAGTGATCGATGCGTCTGAATTAACACCAGCCGTGACAACTTAC  1140
ValThrGluLysProGluValIleAspAlaSerGluLeuThrProAlaValThrThrTyr   380
```

FIG. 2 (CONT.)

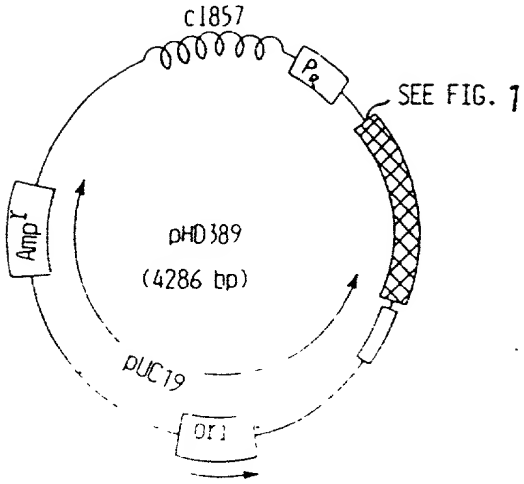
```
    v         v         v         v         v         v
AAACTTGTTATTAATGGTAAAACATTGAAAGGCGAAACAACTACTAAAGCAGTAGACGCA   1200
LysLeuValIleAsnGlyLysThrLeuLysGlyGluThrThrThrLysAlaValAspAla   400

    v         v         v         v         v         v
GAAACTGCAGAAAAAGCCTTCAAACAATACGCTAACGACAACGGTGTTGATGGTGTTTGG   1260
GluThrAlaGluLysAlaPheLysGlnTyrAlaAsnAspAsnGlyValAspGlyValTrp   420

    v         v         v         v
ACTTATGATGATGCGACTAAGACCTTTACGGTAACTGAAATGTAATAA   1308
ThrTyrAspAspAlaThrLysThrPheThrValThrGluMet -  -    434
```

FIG. 2

FIG. 3 SCHEMATIC OVERALL VEIW OF THE PRODUCTION OF PROTEIN L



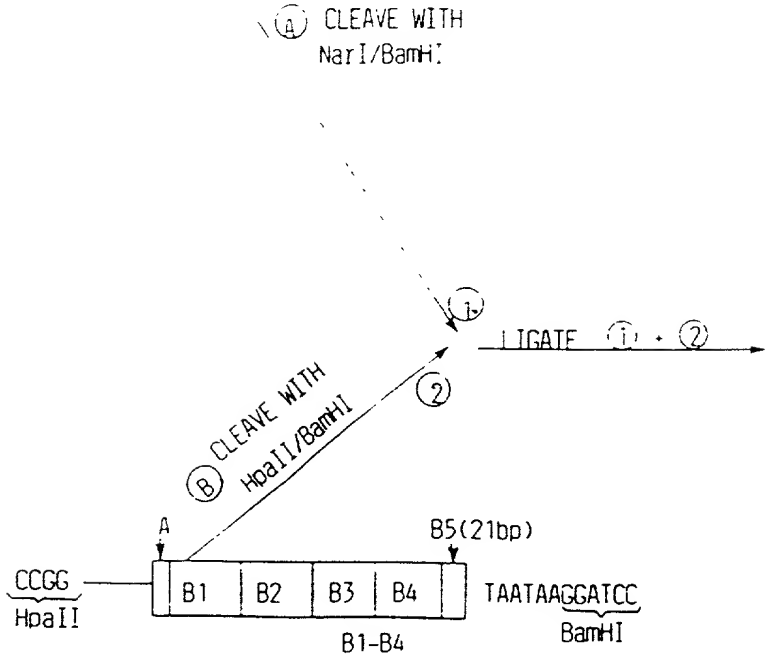
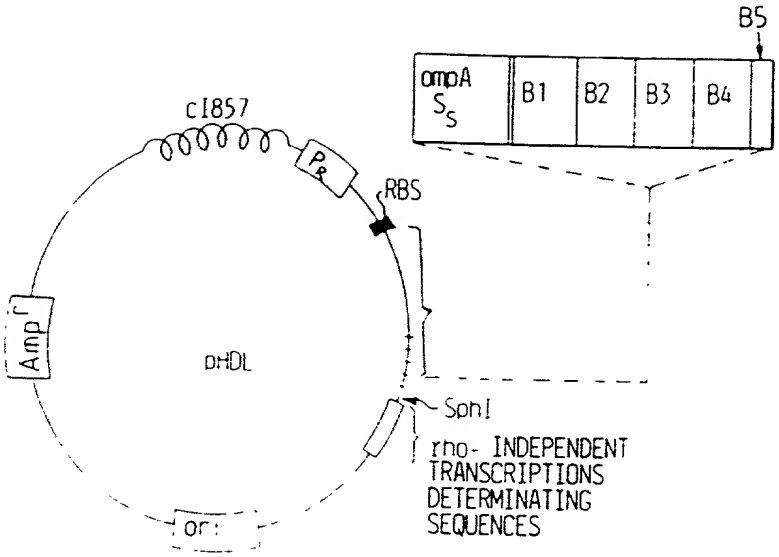


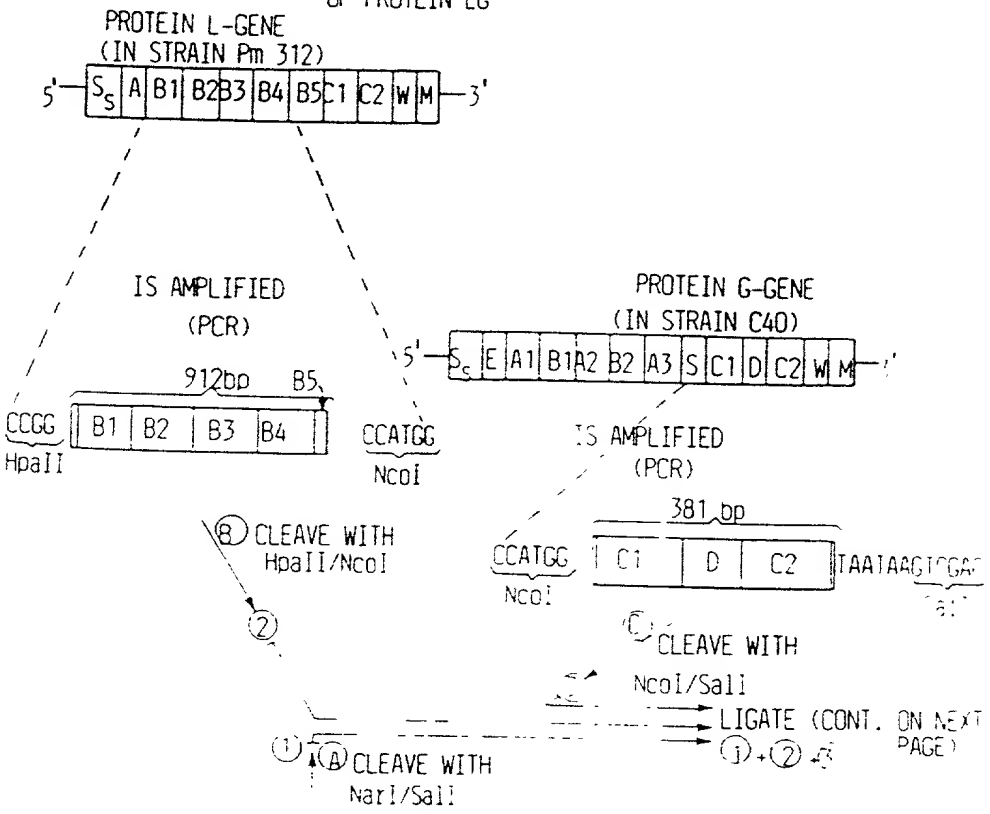
FIG. 3(1)



TRANSFORM
TO E.coli
STRAIN LE392

FIG.3(2)

FIG.4 SCHEMATIC OVERALL VIEW OF PRODUCTION OF PROTEIN LG



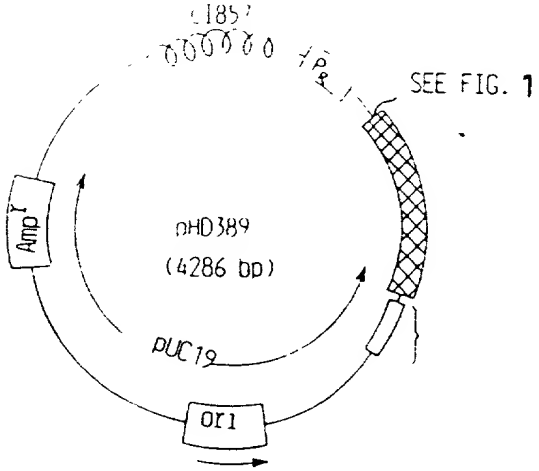
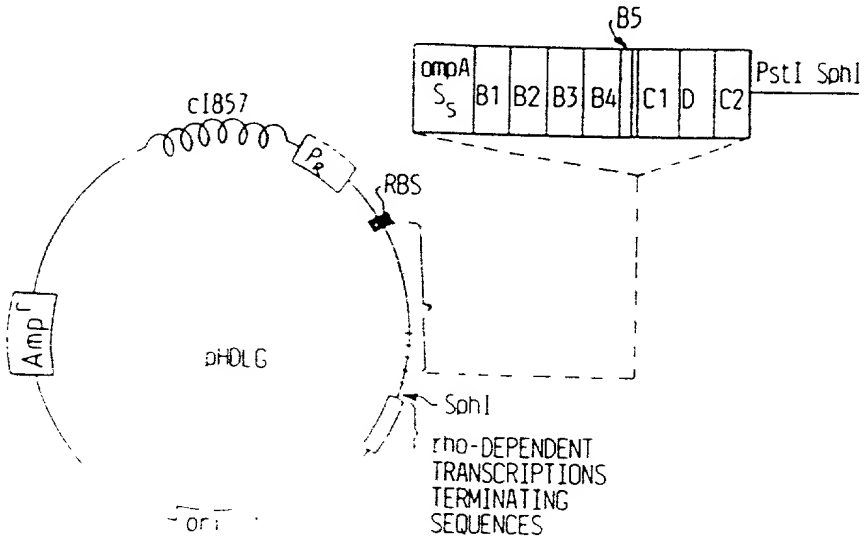


FIG.4(1)



TRANSFORM
TO STRAIN E.coli LE392

FIG.4(2)

FIG.5a SCHEMATIC OVERALL VEIW OF THE PRODUCTION OF PROTEIN LA

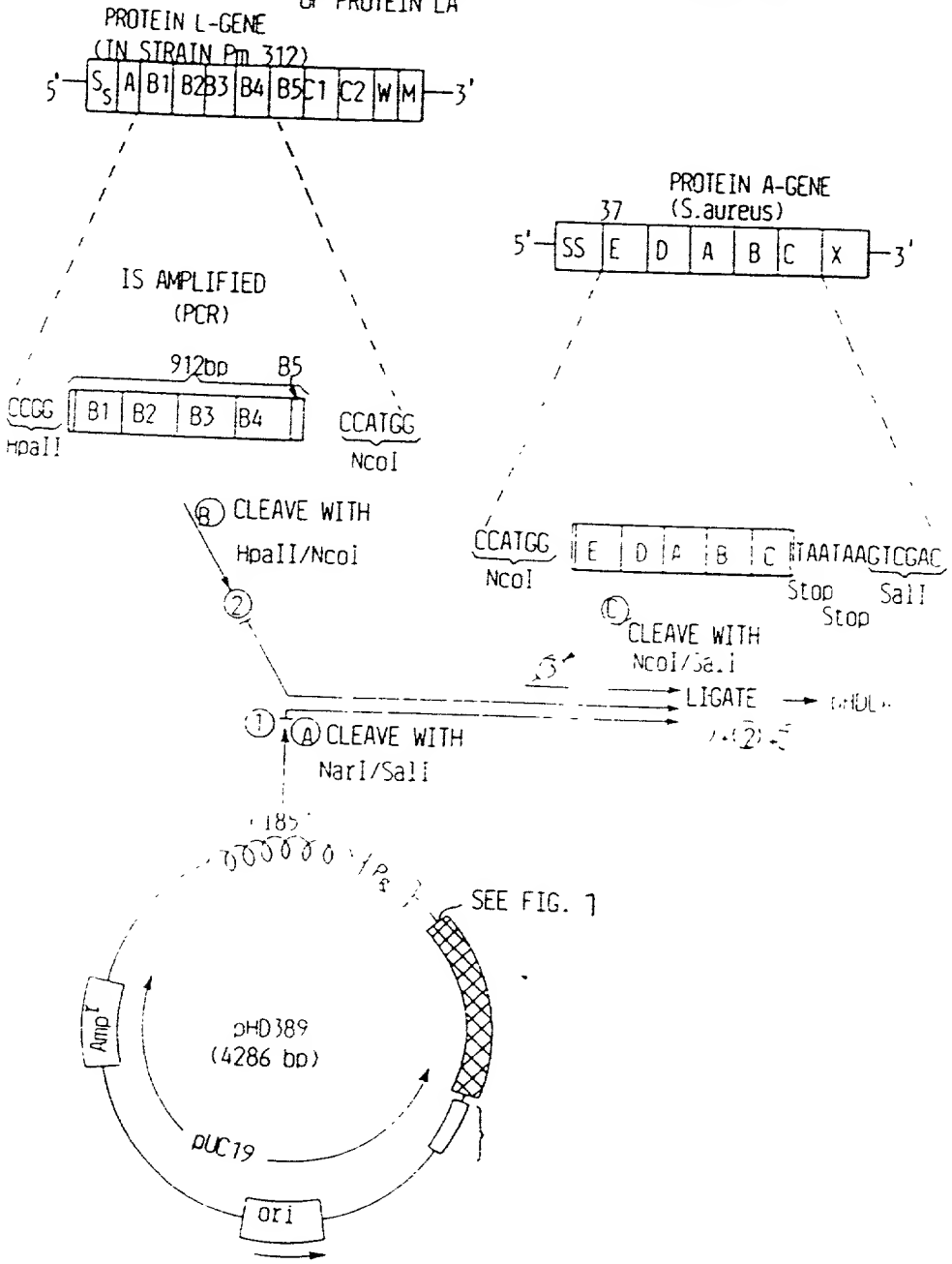


FIG.5b SCHEMATIC OVERALL VIEW OF THE PRODUCTION OF PROTEIN LM

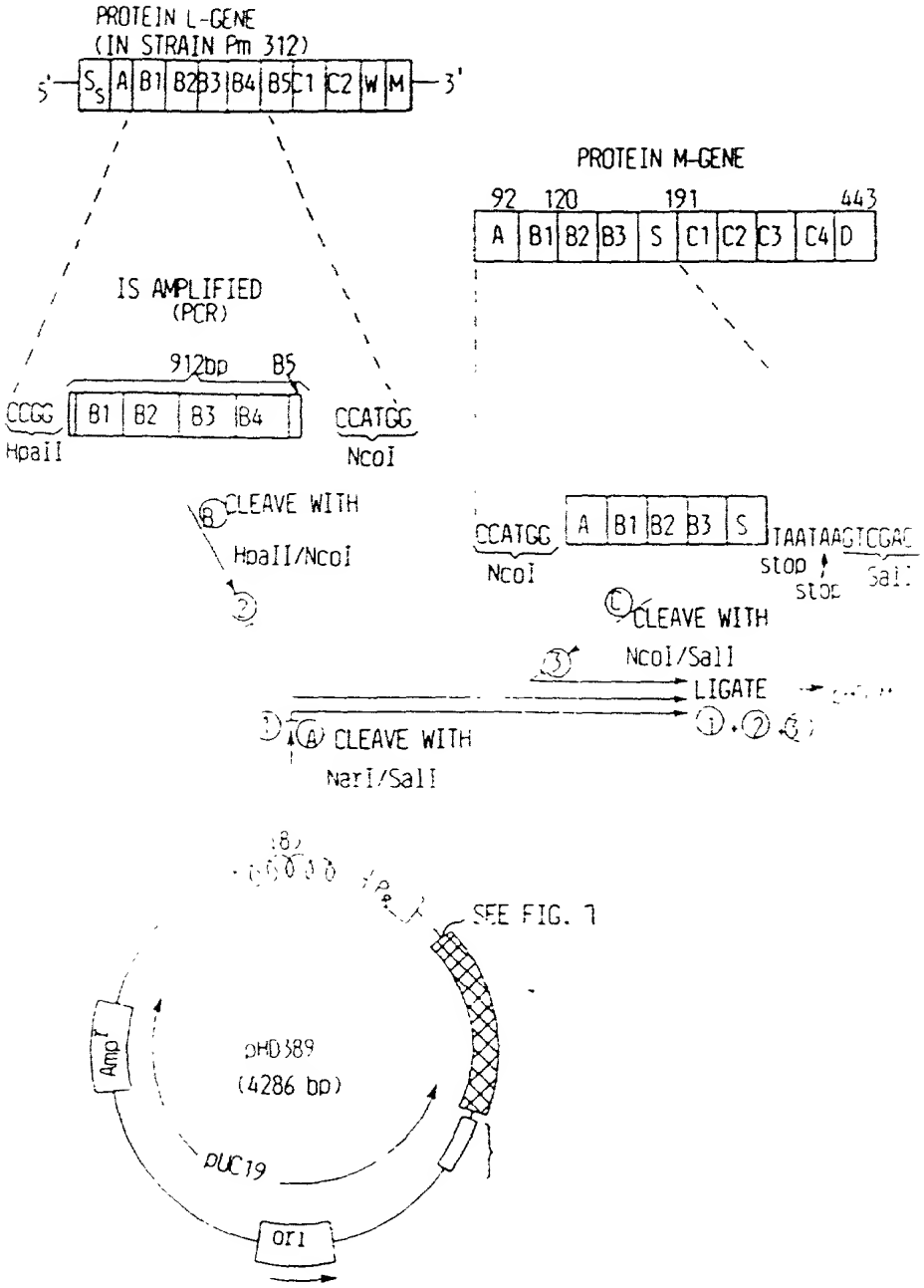
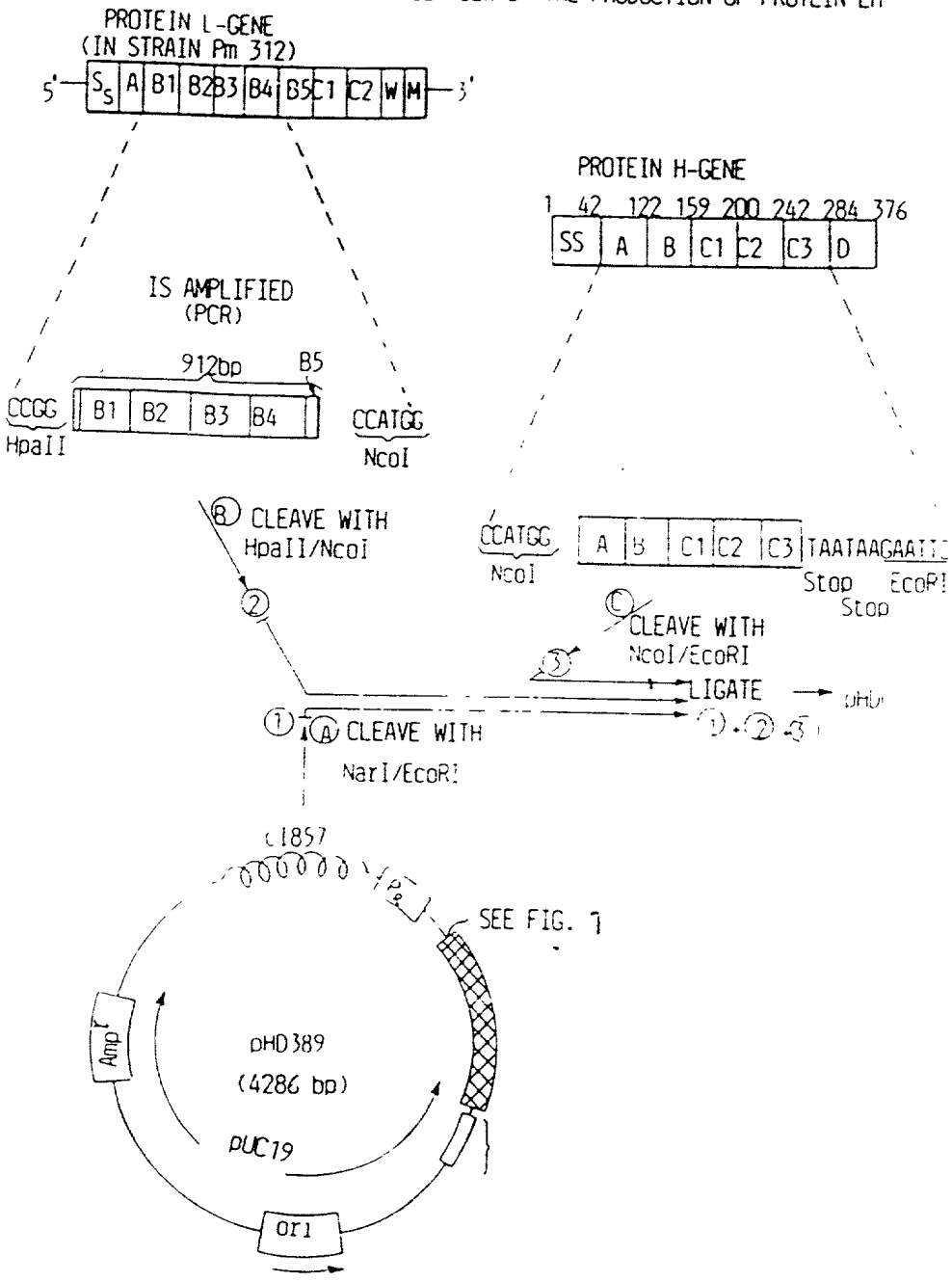


FIG. 5 c SCHEMATIC OVERALL VIEW OF THE PRODUCTION OF PROTEIN LH



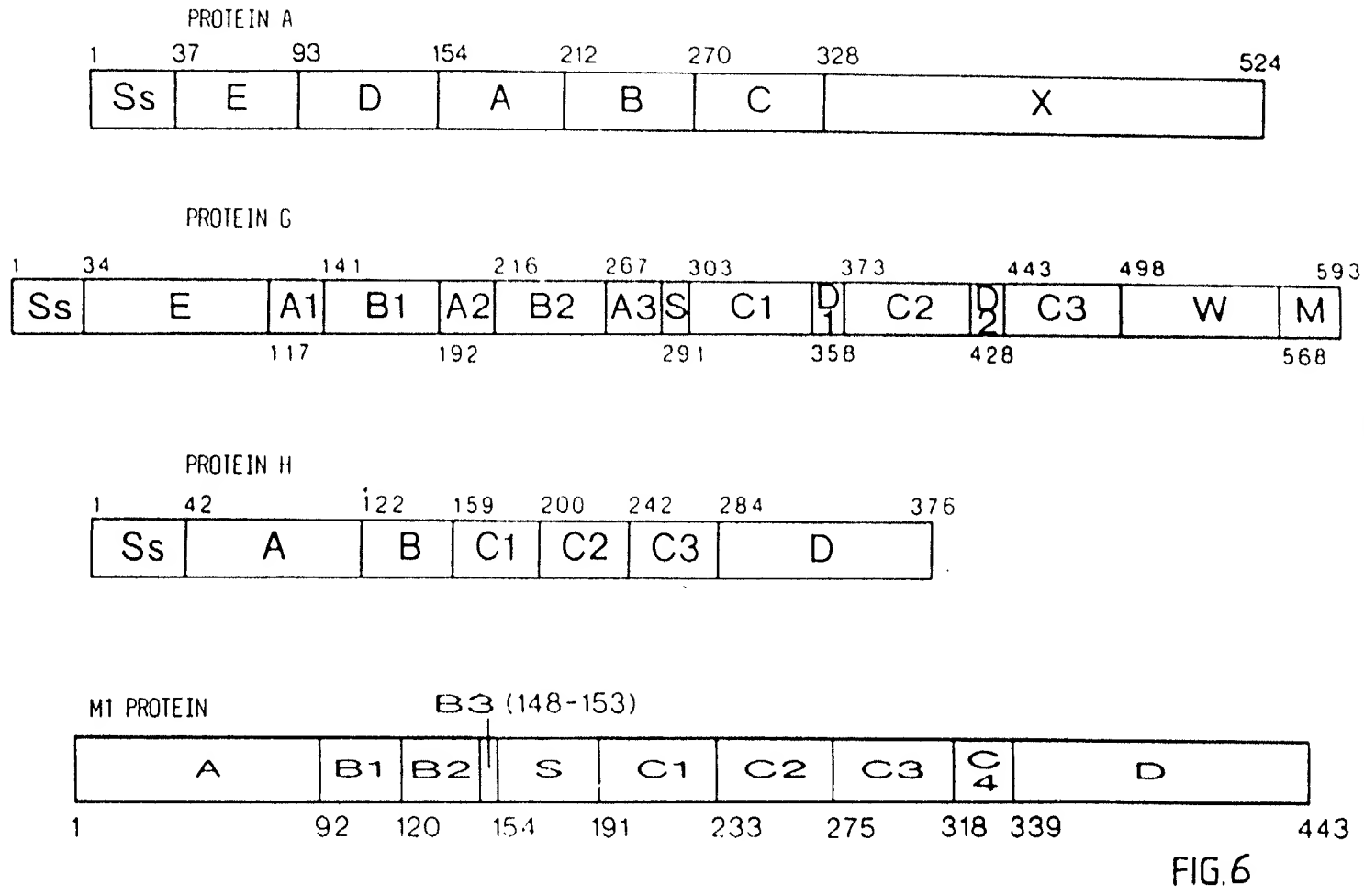


FIG.6

```
AACGGTGATGGTAATCCTAGGGAAGTTATAGAAGATCTTGCAGCAAACAATCCCGCAATA      60
AsnGlyAspGlyAsnProArgGluValIleGluAspLeuAlaAlaAsnAsnProAlaIle      20

CAAAATATACGTTTACGTCACGAAAACAAGGACTTAAAAGCGAGATTAGAGAATGCAATG     120
GlnAsnIleArgLeuArgHisGluAsnLysAspLeuLysAlaArgLeuGluAsnAlaMet      40

GAAGTTGCAGGAAGAGATTTTAAGAGAGCTGAAGAACTTGAAAAAGCAAAACAAGCCTTA     180
GluValAlaGlyArgAspPheLysArgAlaGluGluLeuGluLysAlaLysGlnAlaLeu      60

GAAGACCAGCGTAAAGATTTAGAAACTAAATTAAAAGAACTACAACAAGACTATGACTTA     240
GluAspGlnArgLysAspLeuGluThrLysLeuLysGluGlnGlnAspTyrAspLeu         80

GCAAAGGAATCAACAAGTTGGGATAGACAAAGACTTGAAAAAGAGTTAGAAGAGAAAAAG     300
AlaLysGluSerThrSerTrpAspArgGlnArgLeuGluLysGluLeuGluGluLysLys     100

GAAGCTCTTGAATTAGCGATAGACCAGGCAAGTCGGGACTACCATAGAGCTACCGCTTTA     360
GluAlaLeuGluLeuAlaIleAspGlnAlaSerArgAspTyrHisArgAlaThrAlaLeu     120

GAAAAAGAGTTAGAAGAGAAAAAGAAAGCTCTTGAATTAGCGATAGACCAAGCGAGTCAG     420
GluLysGluLeuGluGluLysLysLysAlaLeuGluLeuAlaIleAspGlnAlaSerGln     140

GACTATAATAGAGCTAACGTCTTAGAAAAAGAGGTTAGAAACGATTACTAGAGAACAAGAG     480
AspTyrAsnArgAlaAsnValLeuGluLysGluLeuGluThrIleThrArgGluGlnGlu     160

ATTAATCGTAATCTTTTAGGCAATGCAAAACTTGAACTTGATCAACTTTCATCTGAAAAA     540
IleAsnArgAsnLeuLeuGlyAsnAlaLysLeuGluLeuAspGlnLeuSerSerGluLys     180
```

FIG. 7 (CONT.)

```
GAGCAGCTAACGATCGAAAAAGCAAAACTTGAGGAAGAAAACAAATCTCAGACGCAAGT   600
GluGlnLeuThrIleGluLysAlaLysLeuGluGluGluLysGlnIleSerAspAlaSer  200
                                190

CGTCAAAGCCTTCGTCGTGACTTGGACGCATCACGTGAAGCTAAGAAACAGGTTGAAAAA  660
ArgGlnSerLeuArgArgAspLeuAspAlaSerArgGluAlaLysLysGlnValGluLys  220

GATTTAGCAAACTTGACTGCTGAACTTGATAAGGTTAAAGAAGACAAACAAATCTCAGAC  720
AspLeuAlaAsnLeuThrAlaGluLeuAspLysValLysGluAspLysGlnIleSerAsp  240

GCAAGCCGTCAACGGCTTCGCCGTGACTTGGACGCATCACGTGAAGCTAAGAAACAGGTT  780
AlaSerArgGlnArgLeuArgArgAspLeuAspAlaSerArgGluAlaLysLysGln*
```

FIG.7 (CONT.)

Amino acid sequence and nucleic acid sequence for protein M1, IgG-binding somewhere between amino acid 1-190.

```
GAAAAAGATTTAGCAAACTTGACTGCTGAACTTGATAAGGTTAAAGAAGAAAAACAAATC      840
GluLysAspLeuAlaAsnLeuThrAlaGluLeuAspLysValLysGluGluLysGlnIle      280

TCAGACGCAAGCCGTCAACGGCTTCGCCGTGACTTGGACGCATCACGTGAAGCTAAGAAA      900
SerAspAlaSerArgGlnArgLeuArgArgAspLeuAspAlaSerArgGluAlaLysLys      300

CAAGTTGAAAAAAGCTTTAGAAGAAGCAAACAGCAAATTAGCTGCTCTTGAAAAACTTAAC      960
GlnValGluLysAlaLeuGluGluAlaAsnSerLysLeuAlaLeuGluLysLeuAsn      320

AAAGAGCTTGAAGAAAGCAAGAAATTAACAGAAAAAGAAAAAGCTGAACTACAAGCAAAA     1020
LysGluLeuGluGluSerLysLysLeuThrGluLysSerAlaGluLeuGlnAlaLysLys      340

CTTGAAGCAGAAGCAAAAGCACTCAAAGAACAATTAGCGAAACAAGCTGAAGAACTCGCA     1080
LeuGluAlaGluAlaLysAlaLeuLysGluGlnLeuAlaLysGlnAlaGluGluLeuAla      360

AAACTAAGAGCTGGAAAAGCATCAGACTCACAAACCCCTGATACAAAACCAGGAAACAAA     1140
LysLeuArgAlaGlyLysAlaSerAspSerGlnThrProAspThrLysProGlyAsnLys      380

GCTGTTCCAGGTAAAGGTCAAGCACCACAAGCAGGTACAAAACCTAACCAAAACAAAGCA     1200
AlaValProGlyLysGlyGlnAlaProGlnAlaGlyThrLysProAsnGlnAsnLysAla      400

CCAATGAAGGAAACTAAGAGACAGTTACCATCAACAGGTGAAACAGCTAACCCATTCTTC     1260
ProMetLysGluThrLysArgGlnLeuProSerThrGlyGluThrAlaAsnProPhePhe      420

ACAGCGGCACGCGTTACTGTTATGGCAACAGCTGGAGTAGCAGCAGTTGTAAAACGCAAA     1320
ThrAlaAlaArgValThrValMetAlaThrAlaGlyValAlaAlaValValLysArgLys      440

GAAGAAAACTAA      1329
GluGluAsn***      443
```

FIG.7

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PCT/SE93/00375

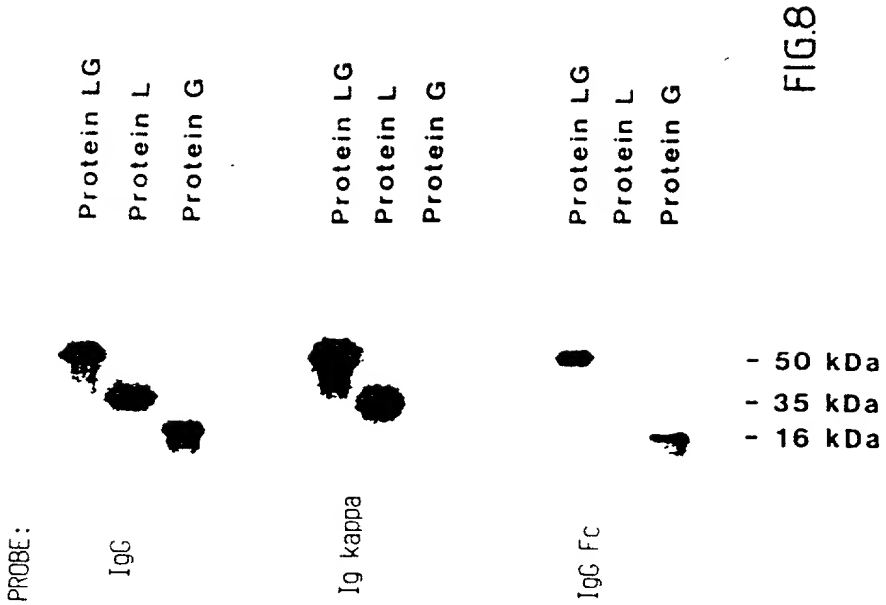

FIG.8

08/325278
PCT/SE93/00375

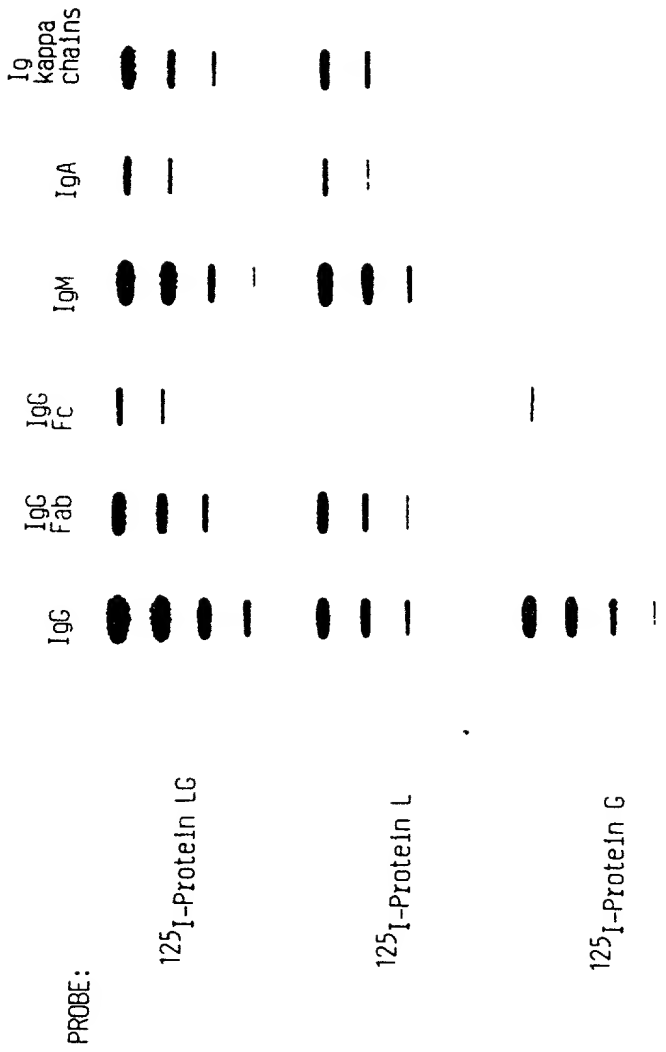

FIG.9

BAR CODE LABEL

U.S. PATENT APPLICATION

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08/325,278	10/26/94	530	1806

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CONTINUING DATA********
VERIFIED THIS APPLN IS A 371 OF PCT/SE93/00375 04/28/93

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FOREIGN/PCT APPLICATIONS********
VERIFIED SWEDEN 9201331-7 04/28/92

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***** SMALL ENTITY *****

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TITLE

PROTEIN L AND HYBRID PROTEINS THEREOF

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